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The Morphology, Cytology and Life-history of *Oodinium ocellatum* Brown, a Dinoflagellate Parasite on Marine Fishes[1].

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(Plates I-IX; Text-figures 1-5).

Contents.

Introduction.

Parasitic dinoflagellates have been known for many years, but it is only recently that a species has been described from fishes. Brown (1931), in a preliminary note, reported a new species, *Oodinium ocellatum*, from the gills and skin of marine fishes, as the cause of a high mortality in the Aquarium of the Zoological Society of London. In a later paper (1934) she extended her observations, but added little concerning the morphology and life-history of the dinoflagellate.

According to Brown (1934), the parasite is found on marine fishes collected from the East and West Indies. In the New York Aquarium, however, the infection has been centered in fishes taken from Sandy Hook Bay and has spread to a few species from Key West, Florida. This is the first record of the parasite from North American waters. None of the East Indian forms present in the Aquarium were found infected.

A number of parasitic dinoflagellates have been described, and much of

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the literature has been reviewed in detail in the excellent monograph by Chatton (1920). Kofoid and Swezy (1921) have pointed out the need for more thorough investigation of these organisms, since many details are lacking in the descriptions of their morphology and life-history. The recent occurrence of *Oodinium ocellatum* in large numbers in the New York Aquarium has afforded an opportunity for further investigation on this species.

The writer wishes to express his thanks to Professor R. P. Hall for the many valuable suggestions and criticisms offered in the preparation of this paper.

Material and Methods.

The parasites were removed for study from the gills of *Chilomycterus schoepfii* and *Spheroides maculatus,* which were dying as a result of the infection. Infected fish could be detected by their actions and also by a pink-tinted mucous secretion on the surface of the body. This pink color is due possibly to waste products of the parasites, much as "red water" is produced by free-swimming dinoflagellates (Meade, 1898; Kofoid and Swezy, 1921; Martin and Nelson, 1929).

Infected gills were fixed in 10% neutral formalin, Zenker's fluid, corrosive sublimate and Bouin's solution. The material was sectioned and after each fixative some sections were stained with Delafield's hematoxylin and others with iron-hematoxylin, eosin being used as counterstain in certain cases. Tissue fixed in Zenker's fluid was also stained with Mallory's triple stain; other material fixed in corrosive sublimate and stained with iron-hematoxylin was counterstained with Van Giesen's fuchsin-picric acid in an attempt to demonstrate fibrils in vegetative stages of the parasites. Most of the hematoxylin-eosin material was destained for the study of nuclear and cytoplasmic structures. In such cases, the rhizoid processes of the attached stages were always completely destained. To overcome this difficulty, a few of the sections were overstained; in this way the rhizoid processes which penetrate the gills were demonstrated.

Parasites showing organelle of attachment in various stages of protrusion were obtained by strongly shaking the gills and allowing the parasites to fall into Schaudinn's and Bouin's fluids. By this method many of the flagellates were fixed before this peculiar organelle could be completely retracted.

In order to obtain division stages a large number of the parasites were washed in sterile sea-water, distributed to several petri dishes, and then fixed at intervals.

The iodine-potassium method, such as employed by Hall and Nigrelli (1931), was used to demonstrate the presence of starch, and in attempts to determine the nature of the small cytoplasmic inclusions described as amyloid bodies by several investigators.

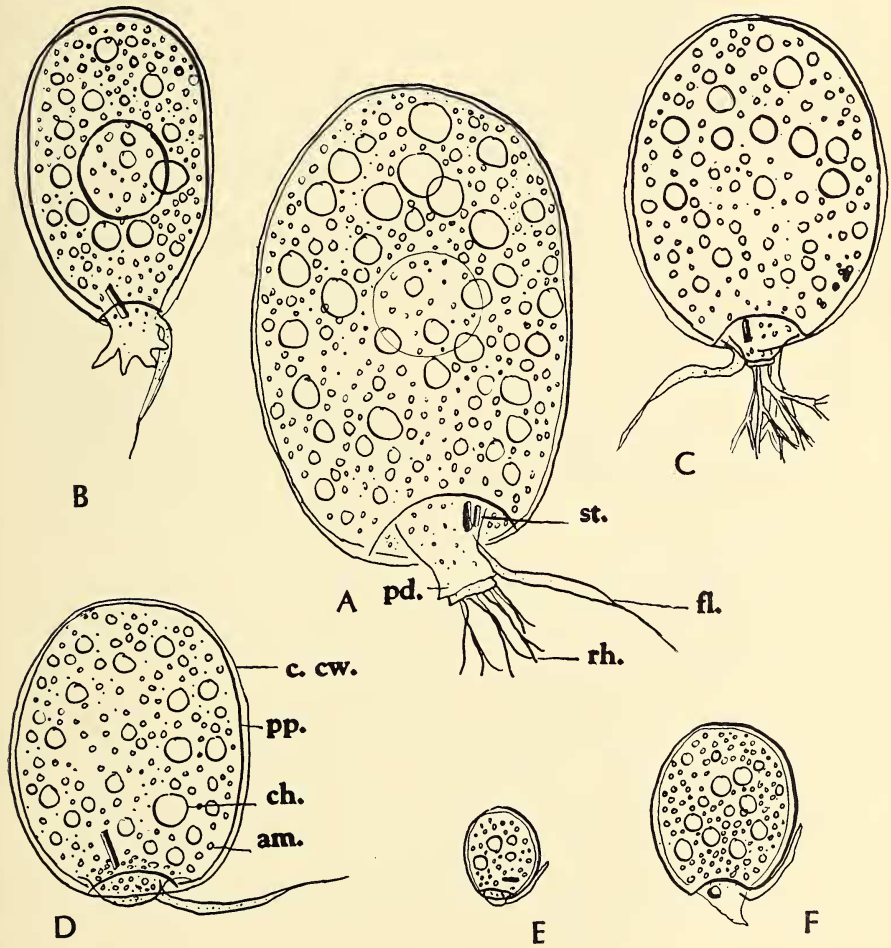
Living specimens were examined under the oil-immersion objective, and many details in the morphology and life-history were observed which would not have been evident in fixed and stained material. In order to check the observations made on mass cultures, single flagellates were isolated in sealed hanging drop preparations, and observed daily. In this way stages in the life-history were traced in pure line cultures.

Experimental infections were attempted in two cases, in one instance with the parasitic stage directly from the gills of an infected host, and in the second with dinospores grown in the laboratory in petri dishes. The results of these experiments are discussed later in the paper. The effects of various temperatures and different specific gravities of sea-water on development were also investigated. Cultures were kept at 12.5°C., 25°C., and

35°C. with the density kept constant at 1.028. In the density experiments, sea-water having an initial specific gravity of 1.028 was gradually evaporated to a density of 1.040. By dilution with normal sea-water, a range was obtained from 1.040 to 1.028. A second series was started with sea-water at a density of 1.028 and, by dilution with fresh water, the range was extended to 1.003. All density readings were taken at the standard temperature of 15°C.

Life-history of *Oodinium ocellatum.*

The parasitic stage of *O. ocellatum* is a pear-shaped organism (Text-fig. 1, A-F; Pl. I, Fig. 1) attached to the gill filaments of marine fishes by means of fine rhizoids. When the organism attains a large size, this method

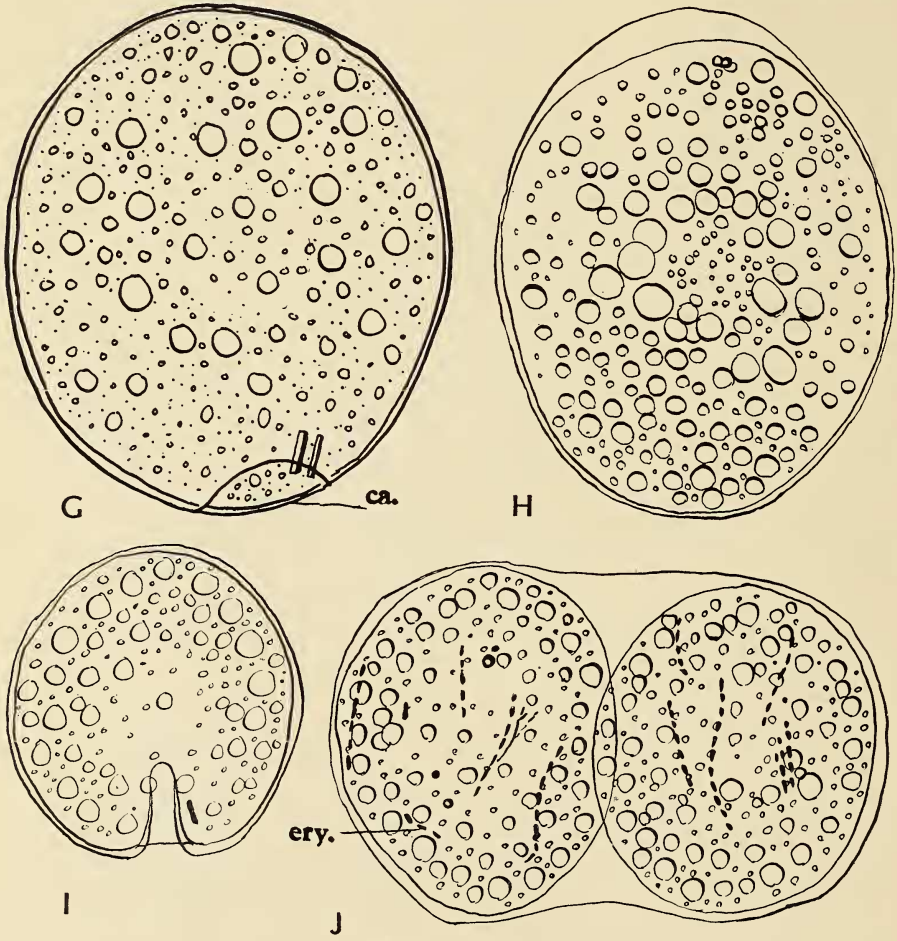


Text-figure 1.

A-F. Camera lucida drawings of the living parasite. x 950. Various stages and sizes of the parasitic form just after removal from the gills. *st.*, stigma-neuromotor complex; *pd.*, peduncle; *rh.*, rhizoids; *fl.*, flagellum; *c. cw.*, cellulose membrane; *pp.*, periplast; *ch.*, chromoplastids; *am.*, amyloid granules.

of attachment apparently becomes mechanically inadequate and the parasite drops off the gills. However, all the organisms, regardless of size, will undergo division once they are removed from the gills. On settling to the substratum, the dinoflagellate takes in water, possibly through the canal present in the peduncle, and increases in volume by one-fourth or more of the original size. All of the organelles, including a broad "flagellum" present in this region, are gradually retracted within the body of the parasite and a cellulose cap is secreted to seal the original opening (Text-fig. 2, G; Pl. I, Fig. 2). When this process is completed, division is initiated.

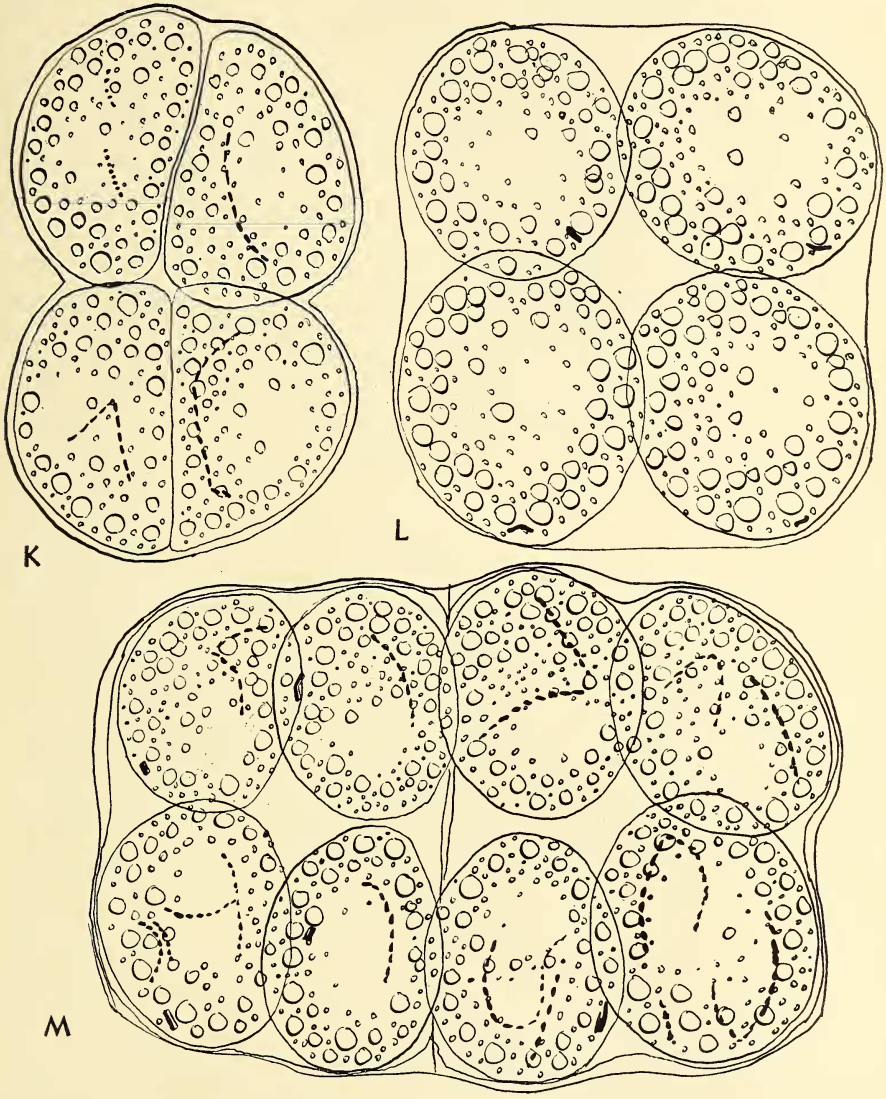
The cytoplasm, at the pole diametrically opposite the region of the peduncle, recedes from the cellulose covering (Text-fig. 2, H; Pl. I, Fig. 3). This is the point at which the first fission will start; therefore, the first division is longitudinal. Succeeding divisions are more or less regular, and



Text-figure 2.

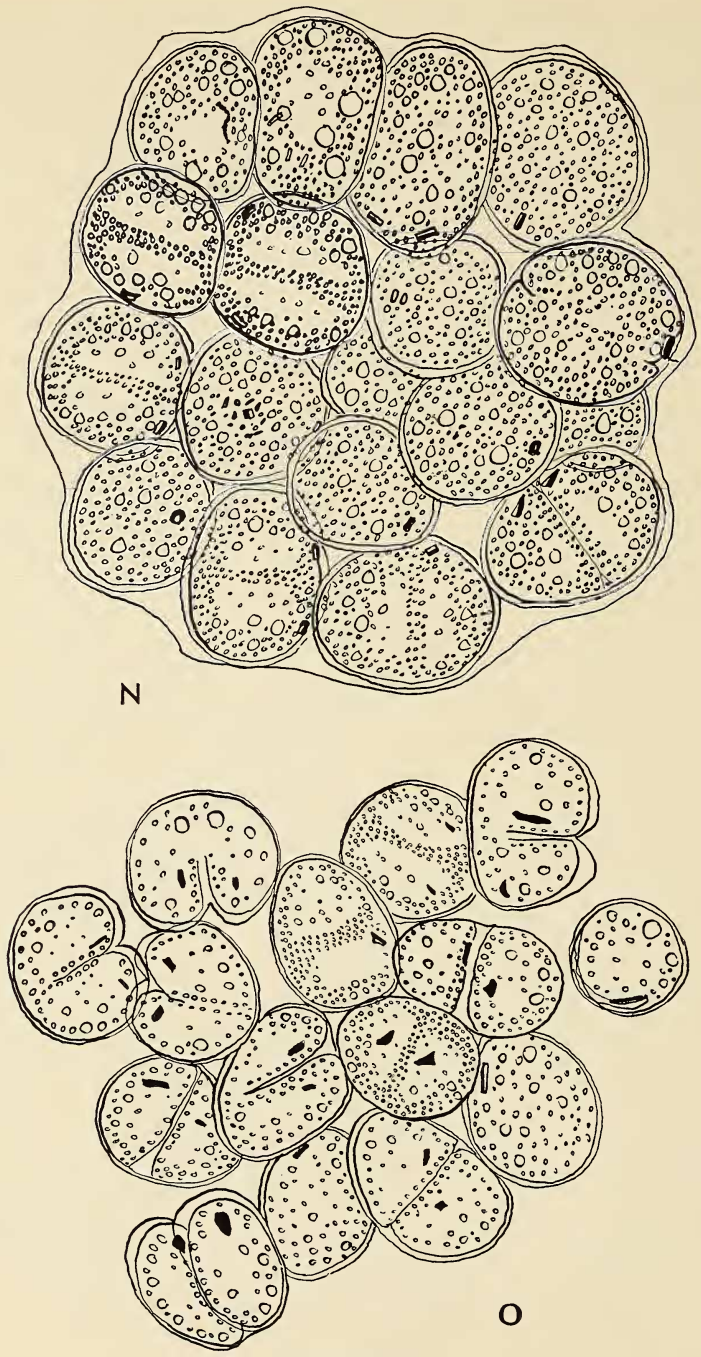
Camera lucida drawings of the living parasite. x 950. G. After imbibition of water a cellulose cap (*ca.*) is secreted. Note the two stigma-neuromotor complex. H. Recession of the cell proper from the cellulose wall at the anterior end. Note absence of both erythrosomes and stigma. I. Stage in the retraction of the polar structures. J. End of the first division; *ery.*, erythrosomes.

at right angles to each other, giving rise to palmella stages of 2, 4, 8, 16, 32, 64, and 128 cells (Text-figs. 2, 3, 4, J-N; Pl. I, Figs. 4, 5). One more palmella division occurs to form 256 minute dinospores. These become flagellated, break through the cyst wall and for a short time are free-swimming naked dinospores but without girdle or sulcus (Text-fig. 5, R, S; Pl. I, Fig. 6). In many cases the dinospores emerge from the palmella before the final division is completed (Text-fig. 5, P, Q). The dinospore then settles to the bottom, secretes a new cellulose covering (Text-fig. 5, T; Pl. I, Fig. 7) and



Text-figure 3.

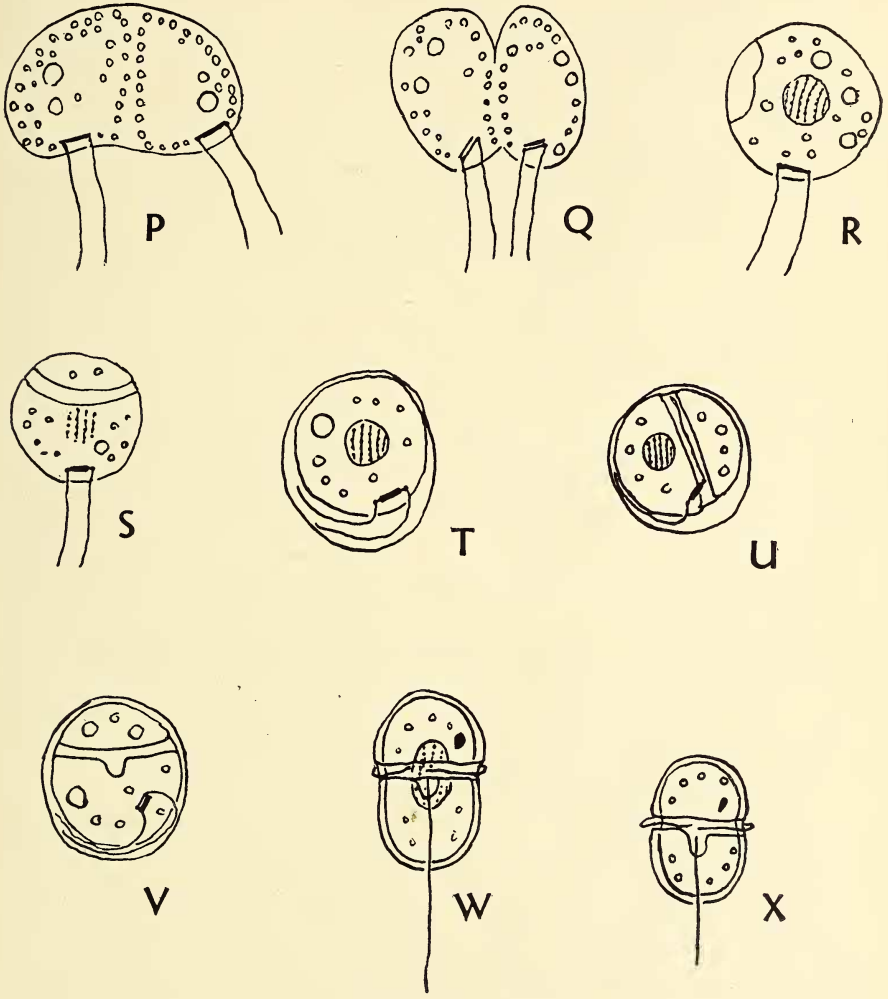
Camera lucida drawings of the living parasite. x 950. K. Beginning of the 2nd cell division. L. End of the 2nd cell division; the erythrosomes have disappeared and in each cell a red bar may be seen but without the "desmose." M. 8 cell stage (flattened).



Text-figure 4.

Camera lucida drawings of the living parasite. x 950. N. Surface cells of a 32 cell stage, some of which show the alignment of the amyloid granules and the beginning of dinospore formation. O. Later stage in dinospore formation.

by certain cytoplasmic changes (Text-fig. 5, S-V; Pl. I, Figs. 8, 9) becomes transformed into a typical peridinian dinoflagellate (Text-fig. 5, W, X; Pl. I, Fig. 10). The latter possesses a well developed girdle with transverse flagellum and a short sulcus from which the longitudinal flagellum passes posteriorly. The metamorphosis of the peridinian into the parasitic stage could not be followed completely. However, evidence shows that the flagella are lost, while the sulcus widens out into a cone-shaped structure (Pl. IX, Fig. 75). From the latter, in all probability, arises the peduncle with its rhizoid processes.



Text-figure 5.

Camera lucida drawings of the living parasite. x 950. P. "Binucleate" naked flagellate stage. Q. Beginning of the final dinospore division. Note the separation of the neuromotor apparatus and the bar-like stigma. R. Temporary free-living, naked dinospore. S. Form in which cytoplasmic differentiation has occurred. T. Rounding up and secretion of a new cellulose membrane. U. Note the orientation of the neuromotor apparatus. V. Cytoplasmic differentiation showing girdle and sulcus. The stigma-neuromotor complex has begun to move anteriorly. W, X. Typical free-swimming *Oodinium ocellatum.*

The Parasitic Stage.

Oodinium ocellatum, (Pl. II, Figs. 11-24) as observed on the gill filaments of the host, measures from 12.4 x 9.9 to 103.7 x 80.5 microns. Average measurements for one hundred specimens taken at random are 61.1 microns in length and 50.1 microns in width. On the other hand, when the organisms round up after removal from the gills, they increase in volume and may measure as much as 150 microns in diameter.

The parasite is surrounded by a firm transparent membrane which, according to Brown (1934), gives a positive reaction for cellulose (Text-fig. 1, c. cw.). The nucleus may be round or oval in shape and in many of the living cells moniliform threads typical of the dinoflagellate nucleus are apparent. The endosome which can be seen in fixed and stained specimens, is not evident in the living material. The cytoplasm contains numerous round chromoplastids (Text-fig. 1, ch.) of varying sizes, usually a pale green in color (Pl. I) and giving the characteristic starch reaction with iodine-potassium iodide solution. When large numbers of the parasites are placed in a test-tube, the entire suspension will give the characteristic blue color when treated with a few drops of the iodine solution. Also present in the cytoplasm are numerous granules which show a purplish color on treatment with iodine. Like Brown (1934), the writer interpreted these as amyloid granules (Text-fig. 1, am.).

In fixed and stained specimens (Pl. II), the cytoplasm appears vacuolated. In Mallory preparations (Pl. II, Figs. 21-24) there are a few red small granules (microsomes?) which seem to be the same as those which stain a light yellow with Van Giesen's method (Pl. II, Figs. 11-16). The nature of these granules is not yet known; they were not observed in living material. The cellulose wall is stained yellow with Van Giesen's, deep blue with Mallory's and a brownish color with iron-hematoxylin. The chromoplastids are usually aggregated around the nucleus as a result of centrifuging. After iron-hematoxylin and eosin, these plastids show small bars or dumb-bell structures which retain the hematoxylin; otherwise they are stained homogenously with eosin. With iron-hematoxylin and Van Giesen's stain (Pl. II, Figs. 14-16), the bars are similarly colored while the rest of each plastid is stained yellow. With Mallory's, the chromoplastids are red or orange with bluish margins while the amyloid granules are light blue (Pl. II, Figs. 21-24). The latter were not evident in other preparations.

One of the interesting features of this dinoflagellate is the organelle of attachment present at the posterior part of the cell (Text-fig. 1, A-F; Pl. I, Fig. 1; Pl. II; Pl. III, Figs. 25-27). This organelle, staining a light blue with Mallory's and yellow with Van Giesen's, is composed of a transparent base or peduncle (Text-fig. 1, pd.) which originates within the cytoplasm proper, passes through a large opening in the cellulose wall and terminates in a pseudopodial tip, sometimes rhizopodial and sometimes lobopodial in appearance, depending on the state of retraction (Text-fig. 1, A-F). It is by means of these "pseudopodia" or rhizoids (Text-fig. 1, rh.) that the parasite anchors itself to the gill tissue of the host. When viewed under oil immersion, the details of this region are clearly visible. In sectioned material (Pl. II), the rhizoids appear as fine threads passing among the cells of the gill tissues. After removal from the gills, these rhizoids become thicker and more blunt in appearance but undifferentiated. In individuals in which a certain amount of retraction has taken place the cytoplasm at the base of the peduncle appears granular. In some cases this granular zone extends anteriorly almost to the region of the nucleus. In Mallory's triple stain preparations this differentiated cytoplasm is stained blue, and in Van Giesen's, yellow. At the base of the peduncle of the attached forms there is a ring which stains red with Mallory's (Pl. II, Figs. 21-24) and yellow

with Van Giesen's (Pl. II, Figs. 11-16). The nature of this structure, which was not observed in the living flagellate, is unknown.

Besides this organelle, there is also present a peculiar broad ribbon-like "flagellum" (Text-fig. 1, fl.), which shows very slow sweeping movements. The "flagellum" is as hyaline in appearance as the rhizoid processes and, except for the movements in living specimens, it is difficult to distinguish this organelle from one of the rhizoids. In fixed and stained specimens removed from the gills both "flagellum" and rhizoid processes seem to arise from the granular cytoplasm at the posterior end of the cell. The "flagellum" apparently can be used in swimming. For example, several specimens were placed in one side of a petri dish and on the opposite side were placed pieces of non-infected gills taken from a killifish (*Fundulus heteroclitus*). Within ten minutes, a dozen young parasites were found attached to the gill filaments. Whether or not this stage of the dinoflagellate moves about on the gills within the branchial cavity of the host has not been determined. In one case the "flagellum" appeared continuous with the canal which extends from the pedunclar region to a dense spherical mass of cytoplasm just posterior to the nucleus. The canal (Pl. II, Fig. 11; Pl. III, Fig. 27) is hyaline in appearance and takes a blue color with Mallory's triple stain. In specimens stained with iron-hematoxylin or Delafield's alone, it appears as a colorless structure, while in those counterstained with eosin, the canal takes on a pink tint. Such a canal was reported by Brown (1934), who likewise was unable to determine the exact relationships of the structure in either living or fixed and stained preparations. However, she also described a club-shaped vacuole (vesicle) connected with the canal in the pedunclar region. In the present material no such vacuole was observed. The stigma (Text-fig. 1, st.), characteristic of *O. ocellatum*, is usually present in the pedunclar region of the parasite and lateral in position. This organelle is composed of a broad red and a thin black pigment bar, between which there is a clear refractile area. Occasionally, there may be two such stigmas (Text-fig. 2, G).

Palmella Stages.

From two to five minutes after the parasites have been removed from the gill filaments, the rhizoids and the "flagellum" are gradually retracted. During the process of retraction, the organism shows a distinct increase in size, probably caused by imbibition of water, since brownian movement of cytoplasmic granules, not previously noticeable, becomes quite evident. After retraction is completed, the cell begins to secrete a layer of cellulose to close the gap (Text-fig. 2, G; Pl. I, Fig. 2). This freshly secreted substance also fixes the enlarged parasite to the substratum (e. g., petri dish, bottom of the tank, coral, etc.). Fission now begins. At room temperature (22° C.) and in sea-water with a specific gravity of 1.028, division is more or less regular and always equal. In living material, fission apparently begins in the cytoplasm diametrically opposite to the point of attachment. In other words, the plane of fission passes through the former antero-posterior axis. Subsequent divisions occur in more or less regular fashion at intervals of about twelve hours, giving rise to 2, 4, 8, 16, 32, 64 and 128 cells. Just prior to the first division, the remnant of the retracted pedunclar processes completely disappears. The ocellus may or may not disappear; as observed in one case, the organelle appeared to be dividing just before the first fission was completed (Pl. I, Fig. 4). Scattered beneath the surface of the cell may be found many red pigmented rodlets ("erythrosomes"), which are constantly shifting position (Text-fig. 2, ery.; Pl. I, Fig. 2). At the end of division these rodlets usually have disappeared (Text-fig. 3, K) and in their place may be found a single red pigment bar near the surface

on each daughter cell. In other cases both ocelli and "erythrosomes" may be present at the same time. The origin and final disposition of these granules was not determined. As the cell divides some of the chromoplastids and amyloid granules pass to each daughter organism.

After the second division, a new cellulose cyst is secreted by each daughter cell at the termination of each fission, so that, when the dinospore stage is reached, each individual is enclosed in its own cyst from which it eventually escapes.

It is interesting to note that if the cells fail to divide at any particular stage within the palmella, certain changes occur in the daughter cells; these have been interpreted as degenerative. The chromoplastids take on a more pronounced yellow color while the pigment granules gradually change from a definite red to a reddish orange and finally to a definite yellow color. The ocelli, however, remain unchanged. These changes appear to be associated with a gradual dehydration of the organisms; indicated by the evaporation of water from the slide.

The amount of fluid present is another important factor for development. In the isolation experiments, if the organism is left in a hanging drop, division may occur up to the 64 or 128 cell stages, depending on the size of the parent cell and the size of the drop. In some cases where the dinoflagellate was accidentally placed at the edge of the drop, division ceased when the four cell stage was reached. However, if any of these organisms are placed in a larger body of medium or in a fresh hanging drop, division will continue even to the formation of dinospores.

Several times there has been observed an interesting process in which, after the first fission, one of the resulting daughter cells fails to undergo further division, while the other gives rise eventually to free-swimming dinoflagellates. This is the type of development ("Palisporogenesis") which occurs normally in the closely related genus *Apodinium*.

Dinospores.

As a rule, in the material under observation, division proceeded in a fairly regular manner to the 128 cell stage and then metamorphosis into dinospores occurred. After flagellation is completed one more division takes place to produce 256 motile individuals. However, under certain conditions (discussed below) dinospore formation by the smaller individuals may be induced at the end of the 8, 16, 32, or 64 cell stages, although never as early as the 2 or 4 cell stage. The factors inducing sporulation appear to be affected by environmental conditions (density of the sea-water, temperature, crowding, etc.) and, contrary to the belief of Brown (1934), are not necessarily related to the size of the original dividing cell.

The formation of the dinospores is an interesting process as observed in living material. Just before the final division, the amyloid inclusions become aligned in the plane of the coming fission (Text-fig. 4, N). Other granules together with the chromoplastids are distributed around the periphery of each cell. Near the surface, orange or red pigment granules of varying shapes and sizes may or may not be present. A red pigment bar with its companion black rod is present near one pole of the cell and in a later stage of development there is at each end of this black rod a granule from which a flagellum arises. In such stages this black rod appears as a "desmose" between the two blepharoplasts (Text-fig. 5, Q; Pl. I, Figs. 6-9).

As observed in permanent preparations (Pl. IX, Figs. 56-74), the neuromotor apparatus arises from what appear to be the centrioles. Certain forms show diplosomes, still within the "centrosphere," from which the flagella are growing out. As will be noted, these centrioles are joined

by a minute fibril. In later stages the fibril increases in length and eventually comes to lie at the periphery of the cell as observed in the living material. In this stage, the flagella appear to be equal in size (Pl. I, Fig. 6). Whether or not the fibril connecting the blepharoplast behave as a paradesmose in fission has not been determined definitely.

By a constant whipping of the flagella, the dinospore frees itself from the cyst wall. It moves about now and then, but it eventually becomes quiet again and then secretes another membrane (Text-fig. 5, T; Pl. I, Fig. 7). In this stage the dinospore is spherical in shape and measures about 15 microns in diameter. The cytoplasm, except for a few chromoplastids and amyloid granules, is clear and non-vacuolated. On the flagellar side, between the cellulose membrane and the periplast (Text-fig. 1, pp.), there is a large space within which lie the flagella (Text-fig. 5, T; Pl. I, Fig. 7). In fixed material only a few chromoplastids are seen in the granular cytoplasm. The nucleus is ovoid or spherical and shows comparatively short and densely stained chromosomes.

Several stages in the transformation of the early dinospore into the typical free-swimming dinoflagellate has been observed (Text-fig. 5, U-X; Pl. I, Figs. 7-10; Pl. IX, Figs. 56-74). Many of the specimens show surface depressions, which presumably will become the girdle and sulcus. These rudiments appear as clear areas, usually on one side of the body. Other flagellates have been found with transverse girdle and a very short sulcus (Text-fig. 5, V; Pl. I, Fig. 9), although the flagella had not yet assumed their final position. In this stage the stigma-neuromotor complex has receded some distance from the surface of the cell. In later stages one of the flagellum comes to lie in the girdle while the other extends posteriorly in the space between the periplast and the cellulose membrane (Text-fig. 5, U).

At the completion of metamorphosis (Text-fig. 5, W and X; Pl. I, Fig. 10; Pl. IX, Figs. 72-74), the free-swimming dinoflagellates are small organisms, measuring about 12 microns in length and about 8 microns in width. The epicone is slightly smaller than the hypocone. The nucleus is approximately central in position and shows the structure typical of the dinoflagellate nucleus. The ocellus lies to the right in the anterior hemisphere. There are a few chromoplastids and amyloid granules, usually arranged around the periphery of the cell. The transverse flagellum extends the full length of the girdle, while the longitudinal flagellum, held more or less rigidly proximally, passes posteriorly along the sulcus. In fixed preparations the fibril joining the blepharoplasts is seen paralleling the longitudinal axis of the cell (Pl. IX, Figs. 72-74).

Although the organisms move about rapidly and are very difficult to observe, it was noted that the transverse flagellum is active in swimming, causing the flagellate to rotate to the right. Lashing movements of the longitudinal flagellum drive the flagellates forward.

Transformation from Dinospore to Attached Stage.

Under natural conditions the free-swimming stage presumably invades the branchial chamber of a fish, becomes attached to a gill filament and metamorphoses into the parasitic type. This metamorphosis has not been traced completely. However, the writer has observed certain changes which appear to be stages in this transformation. In some cases both girdle and sulcus are still present but the flagella have disappeared. Observations on other stages indicate (Pl. IX, Fig. 75) that the rhizoids develop from the sulcal area. In such specimens the girdle is still present, while in the sulcus region there is a finely granular cone-shaped zone of cytoplasm, the apex of which is slightly extruded through an opening in the cellulose wall. Presumably this will eventually form the peduncle with the rhizoid processes.

Nuclear Division.

1. *Interphase.*

The "resting" nucleus of the vegetative stage of *O. ocellatum* is oval or rounded in shape, lying usually near the center of the body. In fifty specimens taken at random, the average size of the ovoid type is 20 x 16 microns. The spherical nuclei average about 16 microns in diameter, the smallest measuring about 12 microns and the largest about 30 microns. The "resting" nuclei of the dinospores are much smaller, averaging about 8 microns.

In the "resting" nucleus of the attached parasites (Pl. II, Figs. 11-24), the chromatin is present in the form of very short, densely staining "threads." With the absorption of water in the early stages of the detached parasites, the nuclei also increase in volume. In these forms, the chromatin is again apparent as short "threads," but staining very lightly with hematoxylin. This staining reaction of the nuclear substance appears to be correlated with this increase in size. Thus, after each division of the palmella (during which stage no more water is absorbed) the nuclear material stains more densely. Similar types of nuclei were reported by Calkins (1899) and Chatton (1914 and 1920) in *Noctiluca* and *Blastodinium* respectively. In both these forms the nucleus is tremendously large and superficially, at least, appears vesiculated. These investigators also reported the fact that the chromatin substance of the nucleus stains lightly with basic dyes.

There is some evidence that the nucleus in *O. ocellatum*, during certain phases of the parasitic stage, assumes an interphase in which chromatin granules (Pl. III, Fig. 25), rather than short "threads," are evident. Chatton (1920) reported this type of "resting" nucleus in young parasites and dinospores of other species of *Oodinium*. Chromatin granules were also reported by Entz (1921) for the interphase nucleus of the free-living dinoflagellate, *Ceratium hirudinella*. Other investigators such as Borgert (1910a) for *Ceratium tripos*, Jollos (1910) for *C. tripos*, *C. fusus* and *C. furca* and Hall (1925 a and b) for *C. hirudinella* and *Oxyrrhis marina* were unable to detect a "resting" stage in which scattered chromatin granules were present. However, no true interphase was observed in any other stage in the life-history of *O. ocellatum*. Once division is initiated, the nucleus assumes a typical prophase appearance in which the chromatin material is present in the form of chromomeres composing beaded chromosomes.

2. *Nuclear Membrane.*

A definite nuclear membrane is present in the "resting" stages of *O. ocellatum*. Chatton (1920) reported the absence of such a structure for the parasitic form of *O. poucheti*, although it was found to be present in *O. fritillaria* and *O. amylaceum*. In the vegetative stage of *O. ocellatum* the membrane is thick, but after the absorption of water it becomes very thin and plastic as is indicated by the indentations caused by the numerous chromoplastids impinging upon it (Pl. III, Fig. 25). During the early stages of division, the membrane persists but eventually it disappears and is reformed in the telophase of the first division cycle (Pl. VI, Figs. 47, 48). However, in the palmella division, the nuclear membrane is not apparent until the telophase of the last division cycle or that division which gives rise to the dinospores.

Calkins (1899) reported that in *Noctiluca* the nuclear membrane persisted during mitosis and disappeared only at the stage when the nuclear plate is formed and the chromosomes were ready for division. In this stage it disappeared in the region between the nuclear plate and the central spindle. Chatton (1914), however, finds that in *Blastodinium* the nuclear

membrane disappeared early in mitosis. On the other hand, Hall (1925 a and b) reported the persistence of the membrane throughout all the stages of the division cycle of both *Ceratium* and *Oxyrrhis*.

3. *The Achromatic Mass in the Resting Cell.*

The achromatic mass or "archoplasm" is present in the sub-nuclear area of the resting cell (Pl. III, Figs. 26-28). It is the differentiated mass of cytoplasm described above. This cytoplasm is stained light blue in Mallory preparations (Pl. II, Figs. 21-24), yellow with Van Giesen's (Pl. II, Figs. 11-16) and brownish with iron-hematoxylin (Pl. II, Fig. 20). In the preparations stained by the first two methods no fibers were evident at this stage. This achromatic mass is large and, in young attached parasites (Pl. II), appears to be continuous with or extends to the base of the peduncle. In larger forms in which retraction of the polar processes has taken place, the two areas, however, are well separated and are connected by the canal described above (Pl. III, Fig. 27).

In addition to the finely granular appearance of this differentiated cytoplasm, densely staining basophilic granules are present (Pl. II). These granules are not unlike the microsomes described by Calkins (1899) in the spheres of *Noctiluca* and by Chatton (1914, 1920) for the spheres of *Blastodinium*. In the division stages of *O. ocellatum* similar granules were found at the fork of the bifurcated strands passing out from the achromatic mass (Pl. VII, Fig. 51).

4. *Mitosis.*

The phenomena of nuclear division in *Oodinium ocellatum* are somewhat complicated, so that the following general summary will help to make the details more clear.

Two kinds of nuclear activity are recognized, one taking place in the first division of the cell, and the other in the palmella, especially after the 8 cell stage, though not necessarily so. In the former, mitosis is not unlike that described by Calkins (1899) for *Noctiluca* and Dogiel (1908) for *Haplozoon*. Such a type is prevalent also in the sporozoans and in certain radiolarians and designated by Belar (1926) as paramitosis. The latter investigator reported such nuclear behavior for *Aggregata eberthi* and *Collozoum inerme*.

In *O. ocellatum*, as in the above species, the "sphere" which lies in the sub-nuclear region elongates during the early stages of nuclear activity. In these stages, the chromosomes are short, thin and stain lightly. Later they appear long, thick and densely stained. The nuclear membrane disappears and the chromosomes become oriented in parallel rows and at right angles to the elongated spindle. The chromosomes split longitudinally while in this stage and from each daughter chromosome mantle fibers pass to both sides of the "sphere." As the "sphere" divides, the chromosomes are gradually drawn upon the central spindle formed and in a still later stage assume a metaphase "plate" appearance. During the anaphase, the chromosomes are drawn towards opposite poles as a result of a further division of the spindle. In the telophase, the chromosomes again become short.

In later palmella stages, mitosis appears to be somewhat different and correlated with the rapidity with which division occurs. In these forms, no orientation of the chromosomes like that described above was noted. However, in late prophase or early metaphase shorter V-shaped chromosomes are present on an elongated spindle; the condition appearing not unlike that found in the metaphase stage of *Syndinium turbo* (Chatton, 1921). In *Oodinium*, however, there is no evidence that the V-shaped chromo-

somes split as a unit, i. e., from the apex of the V and along the axes of the "arms." In later stages of division in *Oodinium*, the chromosomes again appear as a "plate" and the migration of the daughter chromosomes to the poles occurs as in the first mitotic cycle.

5. *Prophase.*

Although one or more endosomes are present (Pl. III, Figs. 25, 26), there is no evidence that this structure takes an active part in mitosis. In large parasites, the endosomes vary in size and shape. They invariably stain lightly and homogenously with hematoxylin.

It is very difficult to delimit the various phases of mitosis in *O. ocellatum* and it is with some hesitancy that the terms employed for the stages of the nuclear cycle in metazoan cells are applied here. The behavior of the nucleus during the early prophase is not completely understood as yet. In Fig. 30 (Pl. IV) the nucleus is elongated, while the chromosomes are still in the shortened phase. The nuclear membrane is still present and within the "sphere" mass the centriole may be seen. In Fig. 31 (Pl. IV) the nuclear membrane has disappeared from the side towards the "sphere." In this stage the short chromosomes of the vegetative nucleus are replaced by long, thin and lightly stained ones. In this case and in others the mitotic figure superficially resembles late anaphase or early telophase, with only one pole of the divided nucleus showing. Thus, Fig. 31 (Pl. IV) is comparable to Calkins' (1899) Fig. 39 (Pl. 42) to which he refers as late anaphase. In the present material, these stages have been interpreted as early phases of mitosis in which the chromosomes have assumed a parallel arrangement but as yet have not thickened. In Fig. 32 (Pl. IV) the nucleus appears as a bilobed structure but the chromosomes are still in the prophase stage. Here, too, mantle fibers are present. Figs. 33 and 34 (Pl. IV) might indicate that the nucleus is forming a C-shaped structure and is beginning to surround the elongated "sphere" (Pl. IV, Fig. 34) somewhat like that reported by Calkins (1899) for *Noctiluca*. However, in so far as could be determined, such is not the case for both parts of each of the nuclei represented are entirely separated.

In many instances the nucleus takes on a sheaf-like appearance (Pl. V, Figs. 38, 39) and although the nuclear membrane has entirely disappeared, the chromosomes are still thin and lightly stained. In these forms, the chromosomes show definite orientation towards the "sphere."

6. *Metaphase and Anaphase.*

In the late prophase or early metaphase, of both the initial and subsequent divisions (up to and including the palmella of the 4 cell stage), the chromosomes are long, thick and rather densely stained (Pl. IV, Fig. 35; Pl. V, Figs. 36, 37, 40). They are definitely arranged parallel to one another and at right angles to the dividing spindle. At this and earlier stages (Pl. IV, Figs. 31, 32), fine mantle fibers pass from the chromosomes to the "sphere." The chromosomes have begun to move into the central spindle formed as a result of the elongation of the "sphere;" during the process some of the chromosomes, moving in opposite directions, pass each other, so that a curious picture is produced. Presumably, as in other paramitotic divisions splitting has been completed by the time the chromosomes begin to move onto the spindle. Once on the spindle, the chromosomes appear as a metaphase "plate" (Pl. V, Fig. 41). There is no evidence, however, that this nuclear "plate" encircles the central spindle as was reported by Calkins (1899) for *Noctiluca* and by Dogiel (1908) for *Haplozoon*. In *Oodinium ocellatum* the separation of the daughter chromosomes is com-

pleted by a transverse fission. This separation has just started in the form represented in Fig. 42 (Pl. V). During the anaphase the daughter chromosomes are drawn to the opposite poles (Pl. VI, Figs. 44, 45; Pl. VII, Figs. 50-51).

In later fission, after the 8 cell stage, this type of nuclear division was not observed. In the early prophase no aligment of the chromosomes was noticed. In later stages, radiating V-shaped chromosomes appear on the spindle (Pl. VI, Fig. 49; Pl. VIII, Fig. 52). The writer interprets such stages as metaphases and the chromosomes are doubling their number by unipolar splitting. As the spindle begins to divide, the chromosomes are straightened out to form the typical metaphase "plate." Division of the chromosomes is completed, possibly, by a transverse fission, much like that described by Hall (1925 a and b) for *Ceratium* and *Oxyrrhis*. Similar type of radiating V-shaped chromosomes was reported by Chatton (1920, 1921) for the parasitic dinoflagellate *Syndinium turbo*. In this form, the chromosome number is doubled by a splitting of the entire V.

7. *Telophase.*

In early telophase the chromosomes become condensed and for a short time maintain their parallel arrangement (Pl. V, Fig. 43; Pl. VI, Figs. 46, 47; Pl. VIII, Fig. 53). In late telophase (Pl. VI, Fig. 47) a nuclear membrane is reformed even before the spindle has been completely obliterated. No evidence was obtained, however, to show that in this first division cycle the reorganized nuclei assume the normal interphase appearance. In all cases seen the chromosomes remained similar to those of the early prophase (Pl. VI, Fig. 48). The nuclear membrane in such forms is thin as in the prophase nucleus at the beginning.

After the 4 cell stage, on the other hand, division is very rapid and the chromosome structure definitely is not altered in this phase but passes into the prophase of the next division (Pl. VIII, Fig. 52). In the final dinospore division, the chromosomes in the late telophase are short, thick and densely stained (Pl. IX, Figs. 56-58).

7. *Achromatic Figures.*

The process involved in the separation of the chromosomes in *O. ocellatum* is an intricate one. As was pointed out above, the achromatic mass, or "sphere" as Calkins calls it, arises from the differentiated cytoplasm in the region just posterior to the resting nucleus (Pl. II, Figs. 12-14; Pl. III, Figs. 25-27). In certain forms, "centrospheres," containing diplosomes described above, are evident (Pl. IV, Figs. 30, 33; Pl. V, Fig. 40; Pl. VIII, Fig. 52) and not unlike those present in *Noctiluca* (Calkins, 1899). From each polar mass, fine strands pass to the periphery, bifurcate and end in the periplast (Pl. VII, Fig. 51). At the fork of each bifurcation is often found a granule (not unlike the microsomes found within the differentiated cytoplasmic mass of the attached parasite), the significance of which is not known. Fig. 32 (Pl. IV) shows what might be the beginning of the fine protoplasmic strands, although the "sphere" has not yet begun to elongate. At the termination of fission, especially at the end of the first nuclear cycle, the achromatic mass is also reformed (Pl. VI, Fig. 48).

It has been shown above that from each of the divided chromosomes minute fibrils (Pl. IV, Figs., 31, 32; Pl. V, Fig. 40) pass towards the center of the "sphere." When the latter elongates to form the central spindle these fibrils converge towards each pole. These are in all probability the radial fibers described by Ishikawa (1899) and the mantle fibers described by Calkins (1899) for *Noctiluca*. The exact relationship of these fibers to those

of the spindle was not determined for *O. ocellatum*. Calkins, although not certain, believed they were nuclear in origin. He showed that these fibers are focused in the centrosome and connect with the chromosomes. Although in *O. ocellatum* fibers were seen passing from the chromosomes, just how far they extended along the spindle could not be ascertained, for in many of these forms no centrosomes were evident. In palmella divisions definite centrioles within centrospheres were often present but no mantle fibers were found. One detached parasite showed two centrospheres in the posterior region of the body connected by minute fibers passing from the differentiated cytoplasmic mass (Pl. VIII, Fig. 55). This would indicate that these fibers at least, are not nuclear in origin.

The centrioles arise as a result of the division of the granules and desmose of the ocellus complex. In one case such a divided structure was seen within a centrosphere-like structure (Pl. VIII, Fig. 54). In the specimen mentioned above, two such centrospheres were present in the granular zone of the posterior part of the cell (Pl. VIII, Fig. 55). Unfortunately, in most of these stages, the nucleus and the surrounding region are partially masked by the large number of chromoplastids and the relationship of this structure to the nucleus could not be determined. However, it is believed that these fibrils focusing towards the centrosphere are in direct connection with the achromatic mass adjacent to the nucleus. Just how they finally attain their final position at the poles of the spindle was not determined.

As just stated, the centrioles arise as a result of the division of the granules and desmose of the ocellus complex. It must be mentioned here, however, that in the living parasite, no granules were observed at the ends of the black pigment bar associated with the red pigment mass of this organelle, although they were quite evident in the early living dinospore stage. However, in all these non-flagellated forms, the red part of the ocellus is always connected to the black portion by means of very minute "fibrils." It may well be that these connecting fibrils condense to form the granules (blepharoplasts) present in the centrosphere. The evidence for the origin of these centrioles from the ocellus complex is further substantiated by the fact that these granules give rise to the flagellar apparatus while still within the centrosphere (Pl. IX, Figs. 64-66).

No definite evidence was obtained as to the presence of a paradesmose such as has been described for many of the free-living flagellates. Hall (1925 a) was the first to demonstrate such a structure in a dinoflagellate. He found that in *Oxyrrhis marina* a paradesmose was formed as a result of a division of the centrosome. In this form the blepharoplasts disappear during late prophase or early metaphase. Therefore in this species, at least, the "desmose" is a centrosome-paradesmose. Kofoid and Swezy (1921) consider the achromatic structure of *Noctiluca* as analogous to the centrosome-paradesmose of other flagellates, since in this form the kinetic elements are extra-nuclear throughout the entire process of mitosis. By the same reasoning, since the centrospheres and spindle are also extra-nuclear in origin, the entire organelle in *O. ocellatum* may be considered analogous to a paradesmose. In this form, however, since it is definitely shown that the centrioles are in fact the blepharoplasts which give rise to the flagella of the dinospores, the desmose may be considered as analogous to a centroblepharoplast-paradesmose of the free-living flagellates.

It must be mentioned here, that in several cases a peculiar fiber was noted, the ends of which terminated in small granules and from each of which two small fibrils pass out to join each of the granules in the centrospheres (Pl. VIII, Fig. 53). If this is a paradesmose, it is possible that with the proper technique this structure can be demonstrated more definitely.

8. *The Mechanism of Mitosis in* Oodinium ocellatum.

There are many theories concerning the mechanism of mitosis in general. Calkins (1899) reported his concept of this process in *Noctiluca*. He states "The nuclear membrane disappears and the mantle fibers connect the ends of the chromosomes with the centrosomes in the spheres. The central-spindle elongates, causing separation of the spheres; the mantle fibers, remaining firm, move with the spheres, dragging the ends of the chromosomes with them. As the central-spindle becomes longer, the chromosomes are more and more separated, until finally the distal ends are separated and the chromosome division is completed." This process seems logical enough, but the question may be asked, what causes the spindle to elongate? The writer believes that the following may throw some light on this question.

In practically all the division stages of *Oodinium ocellatum* a seemingly sol-gel reaction of the cytoplasm was noted. The "sol" phase manifested itself in stained preparations as light and non-granular areas, usually at the poles of the cell (Pl. VII, Fig. 51). The fine strands passing out from the "sphere" seem to be attached to the edges of such zones. A gradual gelation occurs towards the poles of the cell. With this reversal of phase, the strands of the spheres are "pulled" towards the poles, resulting in the elongation and finally the division of the spindle and the separation of the chromosomes on it. At the beginning of division these strands radiate out in all directions. As division proceeds (and the cell elongates) the strands begin to converge more and more towards the poles. When fission is completed, solation once again occurs, the cell rounds up and the protoplasmic strands radiate out in all directions.

It is interesting to note that Calkins (1899) had seen similar strands passing out from the "sphere" of *Noctiluca* but interpreted them as analogous to the astral rays of metazoan cells. Their function in the division processes of the dinoflagellate, however, was not discussed.

Effects of Density of Sea-water on *Oodinium*.

It was shown by Nigrelli (1935) that the monogenetic trematode *Epibdella melleni* MacCallum was unable to withstand sea-water of either a high or low density. A similar experiment was carried out to determine the optimum density necessary for complete development of these dinoflagellates and the effects of densities at either extreme.

Small quantities of modified sea-water with specific gravities ranging from 1.040 to 1.003 (pH range from 8.4-7.1) were made up as described under material and methods. Aliquot portions were distributed to petri dishes and to each dish were added parasites taken directly from the gills of several spiny boxfish. Examination of each dish immediately after the parasites were placed therein showed that all the flagellates were in the vegetative stage (i.e., with the peduncle and rhizoid processes still protruding). At the end of each twenty-four hours a differential count was made of the various division stages present. This was continued for a period of seven days.

The results showed that the optimal density for development to the dinospore stages lies between 1.012 and 1.021. It is interesting to note that within this range all forms may develop into dinospores at the end of the second or third day. At a density of 1.040, division occurred very slowly and dinospores were not formed during the period of our observations. In the majority of specimens, at this density, development reached the 16 cell stage, and only 2% were seen in the 32 cell stage. However, if the organisms were transferred as late as the sixth day to sea-water of lower density (1.028), division continued to the 128 cell stage and dinospores were formed.

At a density of 1.036 division was slow and again a few non-motile dinospores were observed only at the end of the seventh day. In water with specific gravities of 1.034 and 1.032, non-motile dinospores were seen on the fourth and sixth days, and the majority became free-swimming on the seventh day. In densities of 1.030, 1.029 and 1.028 non-motile dinospores were observed as early as the third and fourth days; a few free-swimming dinoflagellates were present on the fifth day and practically all were motile on the sixth and seventh days. In a density of 1.024 many non-motile dinospores were noted on the third day; these became active twenty-four hours later. In the densities of 1.021, 1.018, 1.015 and 1.012 a few non-motile dinospores were observed on the second day, but they did not become free-swimming until the fourth day. In densities of 1.005 and 1.003, the organisms divided very slowly. At the end of the fifth, sixth and seventh days the majority of forms were in the 8 and 16 cell stages and a few non-motile (32 cell stage) dinospores were observed. In fresh water, development continued to the 4 cell stage, and only 18% reached the 8 cell stage. At the end of the experiment (seven days) these 4 and 8 cell stages were transferred to sea-water having a density of 1.009, but no further division occurred. Development in water with a specific gravity of 1.003 was similar to that in fresh water, except that on transfer of the palmellas to a density of 1.009, division continued in a few instances and dinospores were formed. Again, however, most of the palmella reached only the 16 cell stage.

These experiments were carried on at an average temperature of 22° C. In another series, the temperature was varied while the specific gravity was kept constant at 1.028. At 12.5° C., the development was very slow, a few non-motile dinospores being formed at the end of seven days while the majority (60%) were in the 128 cell stage. At this temperature, most of the dinospores did not become free-swimming until the end of the tenth day. At 25° C., the results were somewhat similar to those obtained at room temperature, with a few non-motile dinospores being formed as early as the third day and motile flagellates at the end of the fifth day. At 35° C., development was accelerated considerably at this density. Here, the rate of division was somewhat equivalent to that which occurred in sea-water with a density of 1.015 and at 22° C.

Similar results were obtained by Brown (1934), who found that the organisms were inactive below 10° C. From 10-20° C., the flagellates divided slowly; from 20-25° C., more rapidly, and at about 25° C., sporulation was completed in three days.

These results indicate that the densities most suitable for development lie between 1.012 and 1.028. This is approximately the range observed under natural conditions. However, it is interesting to note that development can occur (at room temperature) over a wide range of specific gravities (1.005-1.036).

At the height of the epidemic in the Aquarium, analysis of the water gave the following readings:

	Density	pH	Temp.	Bound CO_2 mM per liter	Free CO_2 mM per liter
Bay Water	1.0120	7.5	22° C.	2.02	.20
Sea Water	1.0284	8.2	22° C.	2.50	.00

Conditions in the Aquarium were well within the range most suitable for development of *Oodinium ocellatum*. The heaviest infection was found

in fishes in the closed circulation. This was no doubt due to the fact that the infective stages were not washed to the sewer as would be the case in the open circulation or bay water. Therefore, the results obtained in the laboratory compare favorably with the conditions present in the tanks.

Taxonomy.

According to Chatton (1920), one of the first parasitic dinoflagellates to be discovered was *Gymnodinium pulvisculus*, described by Pouchet (1884-1885). Since the specific name had been previously applied by Klebs (1883) to a fresh water type, Lemmermann (1899) renamed the parasite *Gymnodinium poucheti*. In view of the great variety of forms included in the genus *Gymnodinium*, Chatton (1912) erected the new genus *Oodinium* with *O. poucheti* (Lemmermann, 1899) as the type species. The genus is defined by Chatton (1920) as follows: "Dinospores à hémisphère antérieur beaucoup plus développé que le postérieur. Pas de pigment Xantho-chlorophyllien, mais un lipochrome. Formes végétatives fixée par un tronc absorbant fibrillaire. Parasitisme blastotrophe. Pas de scissiparité simple. Sporogénèse intervenant après libération du parasite à produits, homodynames épars." Chatton gave this genus, as he did for many other genera of parasitic dinoflagellates, family (Oodinidae) ranking under the sub-order Gymnodinida. However, both Kofoid and Swezy (1921) and Calkins (1926) relegated all the known parasitic genera to a single family, Blastodinidae Chatton, 1906. In this family, they included the following genera: *Schizodinium* Chatton (1912). *Blastodinium* Chatton (1906). *Apodinium* Chatton (1907), *Parapodinium* Chatton (1920), *Chrytriodinium* Chatton (1912), *Paulsenella* Chatton (1920), *Haplozoon* v.Dogiel (1906 a) (= *Microtoeniella* Calkins, 1915), *Oodinium* Chatton (1912), *Syndinium* Chatton (1910 a), and *Trypanodinium* Chatton (1920). However, Kofoid and Swezy (1921) failed to include *Haplozoon* and, furthermore, they included as true dinoflagellates the genera *Ellobiopsis* Caullery (1915) and *Paradinium* Chatton (1910), two forms which, according to Chatton (1920), are perhaps not dinoflagellates but were provisionally placed in the sub-order Cryptomonadinea because of their cryptomonad-like characteristics. More recently, Reichenow (1930) classified all these forms under the family Gymnodiniidae and added the genera *Endodinium* Hovasse (1922) and *Merodinium* Chatton (1923) to the group of parasitic dinoflagellates.

The genus *Oodinium*, according to Chatton (1920), contains the following species: *O. poucheti*, from the tunicate *Oikopleura dioica; O. amylaceum* (Bargoni, 1894), occurring on *Salpa mucronata* and *S. democratica; O. fritillaria* Chatton (1912) from *Fritillaria pellucida*, and *O. appendicullariae* (Brooks and Kellner, 1908) from the acidian, *Oikopleura tortugensis*. Other species of doubtful identity but temporarily placed by Chatton in the genus *Oodinium* are forms described by Dogiel (1910) as *Gymnodinium pulvisculus* from the annelid *Alciope* sp. and one form that Chatton (1920) has observed on the pteropod *Criseis acicula*. Kofoid and Swezy (1921), in their short discussion of parasitic forms, refer to a species as *Oodinium parasiticum* (= *Gymnodinium parasiticum* Dogiel, 1906). According to Chatton, *G. parasiticum* Dogiel is synonymous with *Chytriodinium parasiticum* (Dogiel).

The life-history and morphology are not completely known for any of the above species of *Oodinium*. In *O. poucheti*, according to Chatton (1920), the parasitic forms are large in size (150-200 microns), ovoid or spherical, without groove or flagella. The cytoplasm contains numerous minute yellow lipochrome granules more or less evenly dispersed. The vesicular nucleus is large. The organ of attachment is made up of a short robust peduncle,

possessing fibrils and terminating in fine rhizoids, which, as in *O. ocellatum*, are capable of retraction. The entire body, including the rhizoids, is surrounded by a cellulose membrane. Reproduction occurs by repeated and equal division, resulting in the development of numerous free swimming, naked dinospores, with a girdle but no sulcus. The details of fission and metamorphosis of the dinoflagellate to the parasitic type are not known.

O. amylaceum was originally described by Bargoni (1894) as one of the Foraminifera and it was Chatton who recognized its true relationships, although palmella and dinospore stages have not yet been described. In this form, the peduncle terminates in an extensive arborization of rhizoids. The cytoplasm contains numerous amyloid granules. Caullery (1906) rediscovered this parasite in the branchial cavity of *Salpa africana* but gave no information on its life-history. However, from the figure he submitted to Chatton (see fig. 4, Chatton, 1920) the rhizoids do not show the characteristic arborization and in all probability it may be another species.

O. fritillaria measures 80 x 130 microns (115 microns in diameter for the round forms). The nucleus in this species is very large, measuring 75 microns in diameter. There are a few yellow lipochrome granules in the cytoplasm. This form differs from the other described species in that the organ of attachment terminates in a broad basal disc measuring 60 microns in diameter. Division and dinospore stages are not known.

O. appendiculariae was first described by Brooks and Kellner (1908) as stages in the development of *Oikopleura tortugensis* and in the same paper (page 93) reported certain forms as a new species of parasitic Foraminifera (*Gromia appendiculariae*). It was Chatton (1920) who pointed out that these attached forms were parasitic dinoflagellates, although no other stages in the life-history are known.

Oodinium ocellatum agrees with the general generic description given by Chatton; since the morphology and life-history of the various species are but imperfectly known, it is difficult, however, to determine specific differences. According to Brown (1931), it "differs from all other members of the genus in the possession of an eye-spot and in its somewhat smaller size." However, there are other differences, some of which are given in the comparison with the type species, *O. poucheti.*

There is no doubt that the dinoflagellate found on fishes belongs to the genus *Oodinium.* Since the generic characters are based on *O. poucheti*, this species may be compared with *O. ocellatum.* The parasitic stage of *O. ocellatum* differs from that of *O. poucheti* in the following characters: (1) presence of chromoplastids and amyloid granules, (2) cellulose cell wall surrounds the body of the parasite, the peduncle and rhizoids being naked, (3) presence of one or more eye-spots, (4) the absence of yellow lipochrome granules, but the presence of red or orange pigment rodlets or globules during certain stages of development and (5) the presence of a peculiar "flagellum" originating in the peduncle. The free-swimming dinoflagellate differs from those of *O. poucheti* in the following: (1) hypocone slightly larger than epicone, (2) presence of a definite, although small sulcus, (3) presence of a few small chromoplastids and amyloid granules in the cytoplasm, (4) red eye-spot and (5) presence of definite cellulose membrane surrounding the entire organism. *O. ocellatum* agrees with *O. poucheti* in the following: (1) the parasitic stage is usually pyriform-shaped organism of rather large size with organelle of attachment composed of a peduncle ending in fine rhizoids, and (2) division is usually equal, giving rise to a palmella with subsequent formation of free-swimming dinospores.

Because the life history of the majority of the species of *Oodinium* is not entirely known, the following tentative key is formulated on the basis of certain characteristics found in the parasitic stage.

Key to the Species of Oodinium *Chatton*, 1912.

A_1 Peduncle terminating in few rhizoids
  b_1 no eye-spot present ..... 1. *O. poucheti* (Lemmermann, 1899)
  b_2 eye-spot present ..... 2. *O. ocellatum* (Brown, 1931)
A_2 Peduncle ending in a broad disc... 3. *O fritillaria* (Chatton, 1912)
A_3 Peduncle ending in an extensive arborization of rhizoids
  4. *O. amylaceum* (Bargoni, 1894)

Such forms as *O. appendiculariae* (Brooks and Kellner, 1908), *Oodinium* sp. (Dogiel, 1910) and *Oodinium* sp. Chatton (1920) are not included because structural details of the type to distinguish them from the species listed in the key are not known. However, on the basis of the little that is known about the parasitic stage, there can be but slight doubt that these forms belong to the genus *Oodinium*.

Incidence of Infection in the New York Aquarium.

According to Brown (1934), the evidence shows that *Oodinium ocellatum* is "indigenous to the warm latitudes and is probably associated with coral reef fishes in Bermuda and the East Indies." The majority of fishes in the New York Aquarium are collected from Key West, Florida, and from Sandy Hook Bay for local species. An occasional specimen reaches the Aquarium from Africa and the East Indies. As was pointed out above, the center of the infection was localized in the spiny boxfish (*Chilomycterus schoepfii*) and the northern or common puffer (*Spheroides maculatus*). The former is commonly found in the more southern waters and only in late summer or early fall does it visit the waters around New York, while the latter is common about our coast from early spring to early winter. Where they go in the late winter is not definitely known.

These species, together with the majority of others caught in local waters, are first kept in bay water at seasonal temperatures and as cold weather approaches they are gradually transferred to heated bay water (22° C.). Since the parasites did not make their appearance in the closed circulation until the early part of December, 1935, the question arose as to what fish served as the original host of the dinoflagellate. Spiny boxfish present in the floor pools (not connected with either the main or warm bay water circulation) showed heavy infection and it is assumed that they brought the parasites with them in their migration from warmer waters and infected other fishes present in the Sandy Hook region at the time.

A list of infected hosts all collected from Sandy Hook Bay follows: Order Acanthopteri (spiny rayed fishes), family Carangidae: (1) *Caranx hippos* (Linn.), common jack, infection mild; (2) *Caranx crysos* (Mitchill), hard-tailed jack, infection mild; (3) *Trachinotus falcatus* (Linn.), round pompano, infection mild; (4) *Naucrates ductor* (Linn.), pilot fish, infection mild. Family Pomatomidae: (5) *Pomatomus saltatrix* (Linn.), bluefish, infection mild; (6) *Roccus lineatus* (Bloch), striped bass, infection mild; (7) *Centropristis striatus* (Linn.), common sea bass, infection mild. Family Sparidae: (8) *Stentomus chrysops* (Linn.), northern porgy, infection mild. Family Sciaenidae: (9) *Cynoscion regalis* (Bloch and Schneider), weakfish, infection mild; (10) *Leiostomus xanthurus* Lacépède, spot, infection mild; (11) *Menticirrhus saxatilis* (Bloch and Schneider), northern kingfish, infection mild. Family Tetradontidae: (12) *Spheroides maculatus* (Bloch and Schneider), northern swellfish or common puffer, infection heavy. Family Diodontidae: (13) *Chilomycterus schoepfii* (Walbaum), spiny boxfish, infection heavy. Family Triglidae: (14) *Prionotus carolinus* (Linn.), Caro-

lina sea robin, infection mild; (15) *Prionotus evolans* (Linn.), striped sea robin, infection mild. Two species collected from Florida were found infected in the Aquarium. These were (16) *Chaetodipterus faber* (Brousset), spadefish, and *Pomacanthus paru* (Bloch), French angelfish. Both of these belong to the family Ephippidae and the former was found to be mildly infected while the latter species died as a result of a very heavy infection.

A few of the species found infected by Brown (1934) are also present in the New York Aquarium, but forms from the West Indies, such as *Angelichthys isabelita* Jordan and Rutter, *Chaetodon capistratus* (Linn.) and *Holocentrus ascensionis* Osbeck, have not as yet shown signs of infection. The cosmopolitan species, *Mugil cephalus*, also present in the Aquarium, is likewise free of the parasites. The East Indian form, *Amphiprion percula* Lacépède, has never shown signs of infection in the Aquarium, although Brown reported that this species and *Psettus argentus* Linn. are always heavily infected and probably introduced the infection into the London Aquarium, indicating that the parasite is present in their natural locality.

It is interesting to mention at this time that swarms of dinoflagellates have been reported from the New Jersey coast by Martin and Nelson (1929) and since "red water" has been seen on many occasions in Sandy Hook Bay by the writer, it is altogether possible that the source of the New York Aquarium infection may be localized in this area.

Transmission Experiment.

Fundulus heteroclitus (Linn.), the common killifish, although present in large numbers in the Aquarium, was never found infected. This is not due to a natural resistance since infections have been induced under experimental conditions. A number of these fish, acclimated to a temperature of 22° C., were placed in two-gallon tanks. In two of the tanks eight fish each were introduced, while a third tank with four fish was used as control. In tank I, a large number of dinospores collected from forms grown in petri dishes were released. In tank II, the gills of a heavily infected spiny boxfish were introduced. Examination of the gills of two killifish removed from tank I on the second day gave positive results. This was to be expected because the infective stages had been introduced in large numbers. Two fish from tank II, in which infected gills with adult parasites were introduced, gave negative results on the second day, and similar results were noted in a fish examined on the fourth day. On the sixth day, however, a fish showed a mild infection. One individual found dead on the eighth day showed a heavy infection of the gills and skin, probably the cause of its death. Since all the parasites undergo a period of division when they are once removed from the gills, it is not surprising to find that infecting the fish in tank II was delayed until the sixth day.

Discussion.

Four definitely recognized species and three additional species of doubtful validity were placed in the genus *Oodinium* by Chatton (1920). Of these, the life-histories of but two species (*O. poucheti* and *O. ocellatum*) are known. The former is parasitic on the pelagic tunicates, *Oikopleura dioica* and *Oikopleura* sp., while the latter occurs on the gills and skin of marine fishes. Of the ten or more genera of parasitic dinoflagellates, the following five are ectoparasitic: *Apodinium* Chatton (1907), *Parapodinium* Chatton (1920), *Chytriodinium* Chatton (1912), *Paulsenella* Chatton (1920) and *Oodinium* Chatton (1912). *Paulsenella* was described as parasitic on

a diatom and *Chytriodinium* as ectoparasitic on copepod eggs. The genera *Parapodinium* and *Apodinium* are found exclusively on pelagic tunicates, while species of *Oodinium* have been reported from tunicates, pteropods, siphonophores and annelids. *Oodinium ocellatum* is the first known dinoflagellate parasite of vertebrates. Brown (1931, 1934) reported this species from the gills and skin of marine fishes of the East and West Indies, while the writer has found that in the New York Aquarium the parasite attacks the spiny boxfish and the common puffer. The former is a warm water species that migrates north on the Atlantic coast during the late summer and early fall; the latter, a local species ranging as far north as the coast of Maine. Other North American species, cited above, have also shown the infection.

In the Dinoflagellida, as in other groups of plant-like flagellates, many species carry on photosynthesis and are thus holophytic, while others are predominantly saprozoic or holozoic in nutrition. As pointed out by Brown (1934), *Oodinium ocellatum* is saprozoic during its parasitic stage and growth continues until the organism severs its connection with the host tissues. In addition to saprozoic nutrition, however, there is some evidence that *O. ocellatum* may carry on photosynthesis. Laboratory cultures were carried successfully in filtered and sterilized sea-water. In addition, the presence of chromoplastids and the demonstration of starch in the flagellate suggests the possible importance of holophytic nutrition in the unattached stages of the life-history. Combined methods of nutrition have been reported for other dinoflagellates, e. g., the holozoic-holophytic type in *Gymnodinium* (Kofoid and Swezy, 1921). The chromoplastids, apparently the structures referred to as "refringent granules" by Brown, are usually a pale to a definite green in color and in degenerating cells take on an ochre coloration. Such chromoplastids have never been recorded for *Oodinium*, although they have been reported for other parasitic dinoflagellates (e. g. *Paulsenella*). Amyloid granules, similar to those observed in *Oodinium ocellatum*, have been described in all the recognized species of the genus.

The ocellus of the parasitic stage of *O. ocellatum* appears to be a true eye-spot (as defined by Kofoid and Swezy), but of a primitive type, intermediate between the simple stigma and the more complex ocellus of the Pouchetidae. In *O. ocellatum* a black and a red pigment bar are associated, but whether or not a definite hyaline lens is present between them could not be determined, although the area between the two bars is highly refractile. For the Pouchetidae, on the other hand, the ocellus is made up of two parts, a refractile, hyaline lens and a pigment mass, the melanosome. The simplest form of melanosome is one in which there is a loose aggregation of pigment granules massed together on one side of the lens. In the more highly developed types, the core of the melanosome contains a red pigment.

The behavior of the more complex types of ocelli during division is entirely unknown. As was mentioned above, the ocellus in *Oodinium ocellatum* may disappear at the beginning of division only to re-appear at the end of the process. In one case, however, the ocellus seemed to be dividing. It was seen as an elongated structure near the surface between the dividing cell and was devoid of the black pigment bar. This phenomenon may not be so rare as the writer's observations might indicate, but there is nevertheless no evidence that the ocellus usually divides in fission. The red pigment rods ("erythrosomes") here observed for the first time in *Oodinium*, usually did not appear in the cytoplasm until the beginning of each division and after the ocellus had disappeared. At the end of division, the red rodlets disappeared, and the elongated ocelli were again formed at the periphery of each daughter cell. This might suggest the breaking up of the red pigment of the ocellus into small rods at the beginning of fission, such as was reported by Hall and Jahn (1929) for the granules of the stigma of *Euglena*. In other cases, however, both ocellus and erythrosomes were present at the

same time. Evidence derived from degenerating cells also seems to indicate that the erythrosomes and the ocelli are not of the same nature. Thus, just prior to cytolysis the erythrosomes would change in color from red to orange-red, orange and finally to yellow while the ocelli always remained red. When cytolysis finally occurred the erythrosomes immediately disappeared while the ocelli separated in two parts (black and red), persisted for some time as such but eventually disappeared in the sea-water. The latter observations agree with the findings of Kofoid and Swezy (1921) for the ocellus of *Erythropsis extrudens.*

The "canal" described by Brown for the parasitic form of *O. ocellatum* was also noted in the stained preparations of the writer's material. This "canal" which is stained lightly with eosin, extends from the peduncle to the achromatic mass in the vicinity of the nucleus, and is perhaps involved in the intake of fluid, as in certain free-living species (Kofoid, 1909). Brown (1934) also regards this canal as homologous with the canal of the free-living dinoflagellates, but the achromatic mass in which it terminates is interpreted by her as the sac-pusule (associated with the canal in free-living forms). The writer was unable to observe the small vesicle described by Brown as emptying into the "canal" and interpreted as a collecting pusule, such as described by Schütt (1895). In the division stages or in the free-swimming forms of *O. ocellatum,* no "canal" or related structures were observed either by Brown or by the writer.

Contractile fibers in the peduncle, such as reported by Brown, were not observed in the present material. Fibers of this sort were reported by Dogiel (1910) for *Oodinium* sp. and by Chatton (1920) for *O. fritillaria.* The latter investigator figured and described a complicated mass of fibers passing from the broad basal disc of *O. fritillaria* and inserting in a granular mass of cytoplasm adjacent to the nucleus. These fibers, according to Chatton, are used in expanding or retracting the disc. Although similar fibers are figured by Dogiel (1910) for *Oodinium* sp., he does not discuss them in his text. On the other hand, he does refer to the "pseudopodial" behavior of the rhizoids. In the present material, the rhizoids behave as "pseudopodia" during retraction. Brown (1934) reported that the fibrils were seen in the "stalk" of the attached parasites of *Oodinium ocellatum.* In the writer's material, no such fibers were noted in attached parasites stained either with Mallory's triple stain or by Van Giesen's method.

In the genus *Oodinium* sporulation has been observed in three species, *O. poucheti, O. amylaceum* and *O. ocellatum,* and the present investigation is the first in which most of the morphological changes have been observed. The general features of sporulation in *O. ocellatum* have been discussed by Brown (1934), who noted that the parasitic form begins to divide, regardless of size, once it is detached from the gills of the host. This observation has been verified in the present investigation. The writer has found that just before division begins there is a sudden increase in size of the flagellate as a result of imbibition of water; this is contrary to Brown's statement that no further increase in size occurred in water. The association of imbibition of water with the division cycle has been reported previously by Entz (1931), who showed that *Ceratium hirudinella* increases in size in this manner immediately following the division and that such a process takes place only at this period in the life-cycle. Later increases are due to real addition of living substance.

With the exception of the members of the two genera, *Oodinium* and *Paulsenella,* the ectoparasitic dinoflagellates sporulate by a process designated by Chatton (1920) as "palisporogénèse." In this type of reproduction, the first division, which is transverse, gives rise to two daughter cells. One of these cells divides repeatedly, eventually giving rise to free-swimming dinospores, while the other merely increases in size at first. However,

when sporulation is completed in the first line, the second of the original cells divides into two cells, one of which gives rise to a second generation of dinospores. As reported elsewhere in this paper, a somewhat similar process has been occasionally observed in *Oodinium ocellatum*. In this case, one of the two original daughter cells gave rise to dinospores at the end of the 32 cell stage, while the second merely increased in size. However, it was not noted if the latter cell also gave rise to dinospores.

Under certain experimental conditions (density and temperature of sea-water) division of *O. ocellatum* is more or less a regular process giving rise to palmellas of 2, 4, 8, 16, 32, 64, 128 and 256 cells. The products of the last division are flagellated dinospores. According to Brown (1934), temperature is the important factor influencing sporulation, and she found the optimum to lie between 23° C. and 27° C. The writer has shown that density of the sea-water is another factor important in the development of the flagellated stages. These results are also of interest in their bearing on the adaptability of *O. ocellatum*. Kofoid and Swezy (1921) have pointed out the delicate nature of the majority of the unarmored dinoflagellates, which are extremely sensitive to handling and to changes in salinity, temperature and pressure. *Oodinium*, on the other hand, is a very hardy type and is more nearly comparable with such forms as *Oxyrrhis*, *Amphidinium* and *Gymnodinium* which Kirby (1934) described from the salt marshes in salinities ranging from 3.5% to saturation.

The morphological changes involved in the transformation from the parasitic type to the free-swimming dinospores have not been recorded previously for any of the species of *Oodinium* or for many of the parasitic dinoflagellates. The movement and alignment of amyloid granules in the binucleate stage of the dinospore, the development of the neuromotor apparatus from the stigma complex, the secretion of a new cellulose membrane, the development of the girdle and sulcus, the migration and orientation of the neuromotor apparatus to its final position in the free-swimming stage are observed here for the first time. Presumably, similar morphological changes may be expected in comparable stages in the life-histories of other species.

The changes in the metamorphosis from the dinoflagellate stage to the parasitic type are also described for the first time. As was mentioned above, the flagellar apparatus is lost and the sulcus widens out to form a cone-shaped structure, the tip of which becomes extruded and possibly gives rise to the peduncle and all its processes.

The development of peculiar structures from the sulcal region has also been reported in other dinoflagellates. The pseudopodia described by Zacharias (1899) for *Gymnodinium*, the prod of *Erythropsis* (Hertwig, 1884), and the tentacles of other species are all developed from an extremely plastic sulcal area. In nearly every case, with the development of these specialized structures, there is a loss of one flagellum, usually the longitudinal one. In *Oodinium*, however, both flagella are lost, and the girdle disappears completely in growth of the more or less pear-shaped parasitic stage.

Nuclear division in *Oodinium ocellatum* is of the paramitotic type and similar in some respects to the process described by Calkins (1899) and Ishikawa (1899) for *Noctiluca*. As in *Oodinium*, Calkins noted the sphere in the resting cells while Ishikawa had only observed this structure in later stages of division. Chatton (1920) has figured (Pl. 1, Fig. 10) a similar differentiated mass of cytoplasm lying adjacent to the nucleus of *O. fritillaria*. However, the significance of this mass was not discussed. In the parasitic stage of *O. ocellatum*, this mass of cytoplasm extends to the region of the peduncle and contains many basophilic granules (microsomes). According to Calkins, these microsomes are first found in a peripheral zone of the sphere in *Noctiluca* and only as division approaches do these granules become concentrated within the sphere. The relation of these granules to

mitosis is not known. In late stages of division of *Oodinium* similar granules are found localized in the "forks" of the bifurcation of the strands passing from the spheres. Chatton (1914, 1920) reported siderophilic granules in the centrosphere of *Blastodinium* but gave no explanation as to what they might be. In this species, the archoplasm or sphere is composed of a granular central zone and a more or less thick, homogenously-staining peripheral zone. Its appearance in the vegetative stage is not reported. However, in certain trophocytes (binucleate stages) centrospheres with astral rays surrounding them are present at opposite poles of large nuclei. Each nucleus contains several nucleoli and is traversed by filaments which he terms plasmodendrites. These are the remains of the nuclear spindle fibers formed by the division of the centrosphere. He points out further that these peculiar structures are formed in sporocytes of all ages, but in the last sporocyst division the centrospheres and achromatic figures disappear and a simple type of "haplomitosis" results. In both *Noctiluca* and *Oodinium*, however, "spheres" are noted throughout all the division stages, and, in *Oodinium* at least, up to and including the last dinospore division. Dogiel (1908) has also seen spheres with central spindle in *Haplozoon armatum* but here again the early stages in the formation of this extra-nuclear structure were not observed.

The behavior of the spindle in *O. ocellatum* during division is somewhat different from that of *Noctiluca*. In the latter, according to Calkins, it elongates as the prophase chromosomes are being formed and at the end of this nuclear phase it consists of two daughter-spheres connected by a "central-spindle." The nucleus elongates and bends to form a C-shaped figure and the central spindle sinks into the depression. The spindle, therefore, lies in the secondary axis of the nucleus, which encircles it, the sphere alone remaining outside. When the nuclear plate is formed, it is wrapped around the spindle like a ring, the chromosomes lying midway between the two poles. A similar arrangement of the nuclear plate was noted by Dogiel (1908) for *Haplozoon*. In *O. ocellatum* no such behavior of the nucleus was observed. In the early stages of division, the achromatic mass elongates. The chromosomes elongate, thicken and lie in parallel rows at right angles to the dividing spindle. As in *Noctiluca*, the chromosomes at this stage are attached to the spindle by means of mantle fibers and as further division of the spindle occurs they eventually form a "plate." However, there is no evidence that this "plate" encircles the extra-nuclear spindle as in *Noctiluca* and *Haplozoon*.

According to Calkins (1899), this behavior of the spindle and the chromosome is a constant feature throughout all the division stages of *Noctiluca*. However, in *Oodinium ocellatum*, it has been observed that, after the 4 cell stage, the arrangement of the chromosomes, prior to forming the "plate," is different. In these forms no early prophases were noted. Here division is more or less rapid and the chromosomes, instead of orienting themselves in parallel rows and at right angles to the elongated spindle, are present as thin, radiating, V-shaped structures within the "sphere." Presumably, these V-shaped chromosomes straighten out as the spindle elongates to form a typical "plate." It is further assumed that the division of the chromosomes is completed by a transverse fission, much like the condition reported by Hall (1925 a and b) for *Ceratium* and *Oxyrrhis*. Chatton (1920, 1921) reported similar radiating V-shaped chromosomes for the parasitic dinoflagellate *Syndinium turbo*. In this form, however, the chromosomes are doubled by a longitudinal splitting of the entire V, starting at the apex and continuing along both "arms." There is no evidence that such a process is present in the late palmella stages of *Oodinium*.

In the free-living dinoflagellates, "spheres" of the type found in *Noctiluca* and *Oodinium* have not been definitely reported. According to Kofoid and Swezy (1921), in certain of Borgert's (1910 a) figures of *Ceratium*

tripos there are suggestions of an archoplasmic structure corresponding to spindle and polar regions. Hall (1925 a) reported a similar condition in *Oxyrrhis*. He states that "In one case (Pl. 28, Fig. 13), a noticeable difference in the two poles of the nucleus is seen; at the anterior end the chromosomes have not yet converged, but seem to extend to a clear area of the cytoplasm. This condition is quite similar to that at the ends of the amphiaster of *Noctiluca* (Calkins, 1899, Pl. 42, Fig. 31), the clear area of the cytoplasm resembling a centrosphere of metazoan cells." In the encysted stage of *Ceratium hirudinella*, reported by Hall (1925 b), a closer similarity to the condition found in *Noctiluca* and *Oodinium* is present. Thus, Hall figures and describes for this form a U-shaped nucleus (pl. 8, figs. 35-36) and in the subnuclear area (pl. 8, figs. 35, 37, 38 and text-fig. D, 1-6) may be seen a differentiated mass of cytoplasm that strongly suggests the "sphere" of the resting and early stages of division of *Oodinium ocellatum*.

The origin of the chromosomes is also different in *O. ocellatum* and *Noctiluca*. In the resting cell of the attached parasite, the chromatin is present in the form of very short, densely staining "threads." When the unattached individual takes in water, the nucleus and the cytoplasm both increase in volume. In such forms, the short "threads" apparently lose their ability to stain densely. In the prophase, long, thin and lightly-staining chromosomes are present. In later prophase and metaphase, the chromosomes are long, thick and more densely stained. In *Noctiluca*, according to Calkins (1899) and Ishikawa (1899), the chromatin is contained in large endosomes, which are referred to as chromatin reservoirs, each of which breaks up into a mass of chromomeres. These collect in chromosome strings or "chromospires." However, the "resting" nucleus of *O. ocellatum* is more like the condition found in the majority of the free-living dinoflagellates. In such forms as *Ceratium* and *Oxyrrhis* (see Lauterborn, 1895; Borgert, 1910 a and b; and Hall, 1925 a and b) the chromatin of the interphase nucleus is present not in the form of disconnected granules (as reported by Entz, 1921) but as chromomeres combined into distinct chromosomes. However, in certain of the recently detached stages of *O. ocellatum*, following the retraction of the polar processes, what appeared to be disconnected granules were observed. Such an appearance was found only at this stage. Chatton (1920), on the other hand, reported that in *O. poucheti*, granular chromatin was present in young parasites and free-swimming dinospores.

In *O. ocellatum*, "centrospheres" containing diplosomes were often noted, especially in certain division stages of the palmellas. Similar centrioles were reported by Calkins for *Noctiluca* during metaphase and anaphase stages, and appeared to be the focal points of the mantle fibers. According to this investigator, "The centrosomes, possibly, come from the nucleus, where, during the resting stages, a small, deeply staining granule can be easily distinguished from the chromatin. This granule disappears during the early stages of chromosome formation." It later appears in the "sphere."

In *O. ocellatum* there is some evidence which indicates that the centrioles are not nuclear in origin, but rather arise from the black pigment portion of the ocellus complex. This is supported mainly by the fact that in living dinospores the flagellar apparatus is intimately associated with the red pigment bar of the ocellus, and in stained specimens, the diplosomes while still within the sphere were seen giving rise to the flagella. In early stages (just after retraction of the polar processes) two elongated bars with granules at each end were seen within a clear area in the posterior region of the body and strongly suggesting the appearance of the diplosome structure. In some cases, two diplosomes were found in the same region, each of which seem to be connected by fibers coming from the "sphere;" unfortunately this area was masked by numerous chromatoplastids so that

the details and relationships of these fibers to the sphere and the nucleus could not be determined. However, it may be pointed out here, as Calkins suggested, that it is very easy to mistake these centrioles for microsomes present in the same region. Ishikawa (1899) reported a single large centrosome for *Noctiluca,* much like the structure seen by Chatton (1920) for *Blastodinium.*

Ever since the early work of Kofoid and his students (Kofoid and Swezy, 1915 a and b, and Kofoid and Christiansen, 1915 a and b), the neuromotor apparatus of flagellates and its behavior during mitosis has received much attention. Jollos (1910) was one of the first to figure such an organelle for dinoflagellates. He showed that in *Gymnodinium fucorum* the apparatus consists of two blepharoplasts, two flagellar rhizoplasts passing from the blepharoplasts to an extra-nuclear granule, and a rhizoplast connecting the latter structure with the endosome. However, the behavior of this neuromotor system during mitosis was not traced. Chatton and Weil (1924) also reported a neuromotor apparatus for *Polykrikos schwartzi.* In this form two blepharoplasts were present, each of which gave rise to two unlike flagella. The blepharoplasts, in turn, were connected by rhizoplasts to granules of a "desmose" found just outside of the nuclear membrane. Here again, the complete behavior of this system of fibers was not followed. Hall (1925 a and b) showed that in *Oxyrrhis* and *Ceratium* the neuromotor apparatus was similar to that reported by Jollos, especially the one found in *Oxyrrhis.* This investigator (Hall), however, was able to follow the behavior of this system of fibers throughout division. He found that a typical paradesmose (centrosome-paradesmose type) was formed between two daughter centrosomes as they drew apart in the prophase and eventually disappeared in the late anaphase. However, just before the end of the prophase, the blepharoplasts disappear and each centrosome gives rise to new flagella. Entz (1928), from observations on living and stained material, found that in *Gonyaulax polygramma,* the flagella ended in two blepharoplasts, but no connection was observed with the centrosphere lying near the nucleus.

In the present material, no neuromotor apparatus was observed in the parasitic stage, although, as discussed above, a broad "flagellum" is present. During division "diplosome" centrioles within centrospheres are often found. In later stages of development (dinospores) these centrioles give rise to flagella. In living material the centrioles, together with their "desmose" and flagella, are intimately associated with the ocellus. Therefore, there is little doubt that the centrioles and the blepharoplasts are identical.

Although no definite paradesmose was observed in *O. ocellatum* during the early formation of the palmella, a paradesmose-like structure was observed in the late anaphase stage. Here, two sets of centrioles were present on both sides of the massed chromosomes. These centrioles were connected with each other by a long "desmose." In other words, this apparatus appears as a precociously developed paradesmose. Such a rapid division of the achromatic figure, however, has been previously reported. Thus, Ishikawa (1899) described and figured a division of the centrospheres in *Noctiluca miliaris* while the chromosomes were still in the late metaphase.

The centrioles in the present material are of the centroblepharoplast type and the central spindle can be considered analogous to a centroblepharoplast-paradesmose. Kofoid and Swezy (1921) consider the "sphere" of *Noctiluca* as a structure analogous to the centrosome-paradesmose of other flagellates. It is altogether possible, however, in view of the fact that the centrioles in this form have not been traced through the final development of the dinoflagellate, that this structure is similar to the one present in *O. ocellatum.* Chatton (1920) reported a centroblepharoplast in an uncer-

tain form of dinoflagellate (an anhang to an uncertain genus, *Atelodinium*) which he called "les spores a rostre." In these spores definite centrosomes were recognized at each pole of the mitotic figure. In later development, these granules gave rise to an aciculated structure which Chatton called a blepharoplast. However, one of his figures (pl. 17, fig. 193) shows a single flagellum, not connected with this elongated "blepharoplast." In 1921, Chatton reported centrosomes which have rise to flagella in the parasitic dinoflagellate *Syndinium turbo.*

Summary.

Oodinium ocellatum Brown is recorded for the first time from the North American coast. The life-history has been redescribed and additional details, previously unobserved, are recorded.

The life-history includes the following stages: (1) the parasitic stage, a large pyriform organism anchored to the gill filaments of the host; (2) palmella stages, in which fission occurs, develop from the parasitic stage after it drops from the gill of the host; (3) flagellated dinospores; and (4) a typical peridinian stage, with girdle, sulcus, transverse and longitudinal flagella.

The parasitic stage is a large pyriform organism surrounded by a cellulose membrane. It possesses chromoplastids, amyloid granules, ocellus and an organelle of attachment composed of a peduncle, "flagellum," and fine rhizoid processes by which the parasite anchors itself to the gill filaments of the host. The ocellus is a characteristic morphological feature of this species. It is composed of a red or orange pigment bar connected to a thinner black pigment bar by means of minute fibrils. The space between the two is highly refractile, superficially appearing as a bar-shaped lens.

The parasite drops from the gill and immediately increases in size as a result of imbibition of water; the polar processes are retracted and the opening closed by a secretion of a cap.

Fission is initiated at the pole opposite to the peduncle and the first division is longitudinal. Just before each fission, erythrosomes or red pigment rodlets usually appear near the surface of the organism and then disappear at the end of division. The ocellus is usually lost when the erythrosomes are present and reappear when the rodlets disappear.

Under certain densities of sea-water and temperatures, the organism divides, giving rise to palmellas of 2, 4, 8, 16, 32, 64, 128 cells. The 128 cell stage divides once again to give rise to 256 free-swimming dinospores. Experimentally, it has been shown that specific gravity is an important factor concerned in the rate with which dinospores are formed. It also has been shown that dinospores may develop at the end of 8, 16, 32 or 64 cell stages and that this precocious development of flagellates is dependent in some degree upon the specific gravity of the sea-water.

The dinospores, when first released, are without a cellulose covering. In these forms, the neuromotor apparatus is part of the ocellus complex, each flagellum arising from a blepharoplast at one end of the black pigment bar, which acts as a "desmose." Later, these naked dinospores settle to the bottom, secrete a new cellulose covering and gradually metamorphose into a typical peridinian dinoflagellate with a girdle and sulcus. This typical dinoflagellate is the infective form.

In the transformation from the pelagic type to the sessile form found on the gills, the flagella are lost and the sulcus hollows out to form a cone-shaped area, the apex of which is later extruded. It is believed that the polar processes together with the "flagellum" are developed from this highly plastic sulcal region.

The vegetative nuclei of *O. ocellatum* are spherical or oval structures,

containing chromatin in the form of short, densely staining "threads." One or more endosomes may be present, but these structures do not take part in the division process.

When the parasites drop from the gills both the cytoplasm and nucleus increase in volume as a result of imbibition of water. The chromatin in these cells fails to stain densely.

A differentiated mass of cytoplasm is present, which in the attached parasites extends to the region of the peduncle. In these forms, many microsomes are found within this mass. In the unattached parasites, especially after rounding up has taken place, two granular cytoplasmic areas become evident and are connected with each other by means of a "canal." One is localized in the subnuclear region and eventually gives rise to the extra-nuclear spindle; the other is formed as a result of the disintegration of the polar processes after retraction.

During division, the achromatic mass or "sphere" elongates to form a central spindle. An interpretation is reported which may throw some light on the mechanism involved in the division of this spindle and the separation of the chromosomes.

Mitosis is of the paramitotic type. Two kinds of chromosome behavior were noted, one which takes place in the initial mitotic cycle and the other in palmellas after the 4 cell stage.

In the early stages of the first mitotic cycle, the chromosomes are present in the form of long, thin and lightly staining threads. The nuclear membrane disappears, and in later stages the chromosomes become thicker and more densely stained. At this stage they lie in parallel rows and at right angles to the elongated spindle. From the ends of the divided chromosomes, mantle fibers pass into the spindle and as the latter structure continues to elongate, the chromosomes are gradually drawn on the spindle and assume a metaphase "plate" appearance. There is no evidence that this "plate" encircles the extra-nuclear spindle as in *Noctiluca* and *Haplozoon.* The chromosomes continue to move to opposite poles and in early telophase maintain their parallel arrangement for a short time but eventually pass into the prophase of the next cycle. There is no evidence that a "resting" stage occurs at the end of division. In later telophase a thin nuclear membrane is reorganized, in some cases even before the spindle fibers are completely obliterated.

In the later palmella division (after the 4 cell stage), chromosomes typical of the early prophases of the initial cycle were not noted. Instead of lining up at right angles to the spindle, the chromosomes appear within the "sphere" as radiating V-shaped structures. It is assumed the V is the result of a unipolar splitting, and as the "sphere" elongates the chromosomes become straightened out to form the metaphase "plate." At this stage, presumably, the daughter chromosomes are separated by a transverse fission. In the telophase, no nuclear membrane is evident. In such forms, the chromosomes pass directly into the prophase of the next division.

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EXPLANATION OF THE PLATES.

Plate I.

Camera lucida drawings of various stages in the life-history of living *Oodinium ocellatum.* x 950.

Fig. 1. Parasite recently detached from the gills of a host.

Fig. 2. Rounding up and the secretion of a cellulose cap. Note the erythrosomes.

Fig. 3. Stage showing the recession of the cell from the outer membrane. This is the anterior end and the point where fission will start.

Fig. 4. First division of a smaller individual. Note the stigma undergoing division.

Fig. 5. 16 cell palmella stage. Certain cells show the alignment of the amyloid granules prior to the formation of the dinospores.

Fig. 6. Naked dinospore. These forms are devoid of a cellulose membrane. The stigma-neuromotor complex lies at the posterior end of the cell.

Fig. 7. A new cellulose membrane is secreted. There is a space between the covering and the periplast, within which the free ends of the flagella eventually come to lie.

Fig. 8. Transformation of the dinospore to the typical dinoflagellate condition. The stigma-neuromotor complex is oriented so that the transverse flagellum lies in the girdle.

Fig. 9. This form shows the differentiation of the cytoplasm of the anterior region into a girdle and sulcus. The flagellar apparatus has begun to move anteriorly.

Fig. 10. Typical free-living *Oodinium ocellatum.*

Plate II.

Camera lucida drawings of attached parasites. x 950.

Figs. 11-16. Corrosive sublimate fixation followed by iron-hematoxylin and Van Giesen's stain.

Fig. 11. Note the canal extending from the peduncle toward the center of the cell.

Figs. 12, 13. Note the ring in the peduncle. This structure stains yellow with Van Giesen's. Note the "microsomes" within the differentiated mass of cytoplasm.

Fig. 14. Section of a large parasite showing the chromoplastids. Note the densely staining structures within the plastids some of which indicate division.

Fig. 15. Young parasite.

Fig. 16. Section of a parasite through the "sphere." Note the plastids.

Figs. 17-20. Zenker's fixation followed by iron-hematoxylin and eosin.

Fig. 18. Section at the level of the nucleus, showing the short interphase chromosomes.

Fig. 20. Individual showing the pseudo-flagellum. Note the radiating arrangement of the chromomeres.

Figs. 21-24. Zenker's fixation followed by Mallory's triple stain. In these forms the chromoplastids, ring and microsomes stain red; the cellulose wall and the edge of the plastids a deep blue, while the amyloid granules take on a lighter blue color.

Plate III.

Camera lucida drawings of detached parasites. x 950. Bouin's fixation followed by Delafield's hematoxylin and counterstained with eosin.

Fig. 25. Detached parasite after a certain amount of swelling has occurred as a result of imbibition of water. Note the chromatin in the form of distinct granules. The peduncle and the rhizoids are slightly retracted.

Fig. 26. Note the elongation of the endosome. The structure, however, does not take part in the nuclear division of this species. The "sphere" may be seen just posterior to the nucleus.

Fig. 27. Typical detached parasite. Note the canal extending from the peduncle to the "sphere" mass.

Fig. 28. Early prophase. The "sphere" mass in this individual is cup-shaped.

Fig. 29. Early prophase. The nuclear membrane has disappeared and the "sphere" shows some elongation.

Plate IV.

Camera lucida drawings of mitosis in *Oodinium ocellatus*. x 950. Material fixed in Zenker's and stained with iron-hematoxylin.

Fig. 30. Early prophase. The nucleus has elongated, but the chromosomes are still in the shortened phase. Note the "diplosome" in the center of the sphere and the scattered microsomes.

Fig. 31. Early prophase. The chromosomes begin to show their parallel arrangement. The nuclear membrane next to the sphere has disappeared and distinct mantle fibres can be seen passing from the nucleus and converging towards the center of the sphere.

Figs. 32-34. Early prophases. These stages are not completely understood as yet. In Fig. 32, the nucleus is a bilobed structure with both lobes extended upwards; the chromosomes are parallel to each other and mantle fibres can be distinguished. The sphere shows what appears to be the beginning of the protoplasmic strands. In Fig. 33 the nucleus shows a still further separation although the sphere has not begun to elaborate as yet. This Figure and Fig. 34 might indicate that the nucleus is forming a C-shaped structure somewhat like that in *Noctiluca*. However, no trace of the part between the ends could be found.

Fig. 35. Late prophase. The chromosomes thicken and elongate considerably. The sphere has begun to elongate. In this form a remnant of the nuclear membrane is still present, although usually when the chromosomes have reached this stage the nuclear membrane is entirely lacking.

Plate V.

Camera lucida drawings of mitosis in *Oodinium ocellatum*. x 950. Material fixed in Zenker's and stained with iron-hematoxylin.

Fig. 36. Late prophase. The chromosomes are being drawn upon the central spindle which is formed as the sphere divides further and further.

Fig. 37. Slightly earlier stage than Fig. 36.

Figs. 38, 39. Prophase. Many forms were encountered with this sheaf-like formation of the nucleus.

Fig. 40. Late prophase. Superficially this figure appears as a metaphase stage but actually the chrosomes have not begun to migrate onto the spindle. Note the diplosome centrioles to which the mantle fibres can be seen converging.

Fig. 41. Metaphase. The chromosomes are aligned on the central spindle. The final separation has just started in the form represented in Fig. 42.

Fig. 43. Telophase. The chromosomes have shortened and cell division has occurred. The daughter nuclei are still connected by the remains of the central spindle.

Plate VI.

Camera lucida drawings of mitosis. Zenker's fixation followed by iron-hematoxylin stain. x 950.

Figs. 44, 45. Early and late anaphase. Note the central spindle fibers.

Figs. 46, 47. Telophase. In Fig. 47 the daughter nuclei are still connected by the remains of the central spindle and a thin nuclear membrane is reorganized round each nucleus.

Fig. 48. Late telophase. Reorganization of the daughter nuclei has occurred and the "sphere" mass once again reappears in the subnuclear region.

Fig. 49. 4 cell stage. Note centrosphere with diplosomes and V-shaped radiating chromosomes in two of the cells. In the two cells to the right, the separation of the chromosomes has occurred, presumably after the V's have straightened out.

Plate VII.

Camera lucida drawings of nuclear division.

Fig. 50. Early anaphase. 4 cell stage. After the chromosomes are arranged upon the central spindle, a transverse separation occurs and the migration to the opposite poles begins.

Fig. 51. Anaphase. Note the processes passing from the poles of the sphere to the periphery. These processes bifurcate and at the forks of the bifurcations may be seen a small densely staining granule.

Plate VIII.

Camera lucida drawings of mitosis in *Oodinium ocellatum.* x 950. Stages showing the V-shaped radiating chromosomes. Note the centriole in Fig. 52.

Fig. 53. Early telophase. The figure to the right is an optical section showing a paradesmose (?).

Fig. 54 shows a divided "desmose" in the posterior part of the cell.

Fig. 55 shows two diplosomes to which mantle fibers (?) converge.

Plate IX.

Camera lucida drawings of dinospores. x 950. Zenker's fixation and iron-hematoxylin stain.

Figs. 56-74. Metamorphosis of *Oodinium ocellatum* to the free-swimming dinoflagellate stage.

Figs. 56-62. Dinospore prior to the final division.

Figs. 56-58. Late telophase.

Fig. 59. Note peculiar spiral formation of the cellulose membrane.

Figs. 60-63. Flagellation.

Figs. 64-66. Note the flagella growing out from the diplosome.

Fig. 67. Orientation of the neuromotor apparatus. Note cytoplasmic differentiation which eventually will form the girdle and the sulcus.

Figs. 68-70. Various forms showing different stages and appearance of the neuromotor apparatus.

Fig. 71. Neuromotor apparatus torn from the cell. Note the blepharoplasts and the connecting desmose.

Figs. 72-74. Cytoplasmic differentiation resulting in the development of the girdle and sulcus. Note the migration of the neuromotor apparatus to its final point in the sucal-girdle junction.

Fig. 75. Stage in the transformation from the free-swimming dinoflagellate to the parasitic type. In this form the sulcus has developed into a cone-shaped structure, the tip of which protrudes through an opening in the cellulose cell wall.